Chitosan Citrate Membranes for Naproxen Delivery

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ABSTRACT

Chitosan citrate, chitosan citrate/glycerol and chitosan citrate/poly(ethylene glycol) blend membranes were prepared by the solvent casting method. Citrate ion was used as the ionic crosslinker. Glycerol and PEG were added to improve flexibility, and sodium chloride to introduce porosity. The primary concerns in the selection of all components were biocompatibility and safety. Chemical structures of the membranes have been investigated by FTIR spectrophotometry, while the surface morphology has been investigated by SEM analysis. Swelling kinetics has been followed gravimetrically. Naproxen, an analgesic and anti-inflammatory agent, has been used as a model drug to test for the drug release properties of the membranes.

It was found out that swelling, drug loading capacity and drug release properties were affected by the composition and the porosity of the membrane. The maximum release percentage was 31% for the membranes with 1% w/v chitosan concentration while the maximum percentage was 25% for the membranes with 0.5% w/v chitosan concentration. All membranes exhibited a burst effect within the first hour. Then a linear release which obeyed zero order release kinetics was observed between 1-6 hours with release rates changing between 1.2%/h to 0.99%/h, except for the sample 7 whose release rate was 0.41%/h between 2-12 hours.

Keywords: Drug delivery, Crosslinking, Chitosan membranes, Citrate, Naproxen

ÖZ

Kitosan sitrat, kitosan sitrat/gliserol ve kitosan sitrat/poli (etilen glikol) karışım membranı çözücü döküm yöntemi ile hazırlandı. Sitrat iyonu çapraz bağlayıcı olarak, gliserol ve PEG ise esnekliği artırmak üzere kullanılmıştır. Porojen olarak sodium klorür kullanılarak gözenekli membranlar hazırlanmıştır. Bileşenlerin seçiminde biyouyumlu ve toksisiteden uzak maddeler olmasına özen gösterilmiştir. Membranların kimyasal yapıları FTIR spektrofotometresi tarafından araştırılırken, yüzey morfolojisi SEM analizi ile incelenmiştir. Şişme kinetiği gravimetrik olarak takip edildi. Ağrı kesici ve anti-enflamatuar ajan olan Naproksen, membranların ilaç salınım özelliğini test etmek için model ilaç olarak kullanılmıştır.

Şişme, ilaç yükleme kapasitesi ve ilaç salımı özelliklerinin membranın gözenekli olup olmamasına ve komposizyonuna bağlı olarak değiştiği gözlemlenmiştir. Maksimum ilaç salınım yüzdesi, 0.5% w/v kitosan konsantrasyonlu membanlarda %25 iken 1% w/v kitosan konsantrasyonlu membanlarda %31'dir. Çalışılan membranlar ilk bir saat içinde patlama etkisi sergilemiştir. 1-6 saat süreleri arasında sıfırıncı derece kinetiğine uyan 1.2%/h - 0.99%/h aralığında doğrusal salınım gözlenmişken, örnek 7 için ise bu salınım 2-12 saatlerinde aralığında 0.41%/s değerindedir.

Anahtar Kelimeler: İlaç salımı, Çapraz bağlanma, Kitosan membranlar, Sitrat iyonu, Naproksen.

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To My Family, Teachers, Friends and Relatives

لعائلتي وأساتذتي وأصدقائي وأقاربي

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Chapter 1

INTRODUCTION

Biopolymer membranes are not only useful in separation technologies for cleaning purposes but also in pharmacy and medicine as biomedical devices for drug release. Biopolymers are from natural origin hence constituting a renewable class of materials. They are also biodegradable and biocompatible, in general. They have good membrane forming properties but they may lack the desired mechanical properties and morphology in many cases. However, biopolymers possess enough chemical functionality which allows modification to tune their properties. Therefore, it is possible to design and produce biopolymer membranes with required properties.

Chitosan is a polymer of natural origin with amine functionality that gives rise to a cationic polymer in acid solution. It dissolves in aqueous hydrochloric acid, acetic acid, formic, oxalic, lactic and citric acids and forms salts with these inorganic and organic acids. It acts as a polycation with a high charge density, owing to the protonation of the amine groups on the chain backbone. It is a weak base with pKa value of around 6.5. Therefore, it is insoluble in neutral and alkaline media [2].

Chitosan salts formed upon dissolution in aqueous acids can be cast in the form of films by solvent evaporation technique. Furthermore, ionic gel formation occurs when

chitosan solutions are mixed with solutions containing of polyfunctional anions. Films, beads, micro and nanoparticles can be formed as a result of physical crosslinking of chitosan chains due to the ionic interactions between positively charged chitosan and negatively charged polyanion. Formation of ionically crosslinked chitosan based beads formed as a result of interaction between chitosan and the tripolyphosphate ion has been studied extensively. Stable, spherical gel beads are formed due to the strong ionic interactions with the pentavalent tripolyphosphate ion [2, 55, 30].

The citrate ion is another potential candidate as a suitable ionic crosslinker for chitosan due to its trivalent nature. A limited number of studies on the formation, properties and uses of chitosan citrate films are available in the literature [30]. These studies reveal that chitosan citrate membranes are more stable than chitosan acetate membranes in aqueous solution and become elastic upon water absorption. They also exhibit porous morphology when freeze dried leading to useful scaffolds for wound healing. Chitosan citrate membranes have been tested for transdermal drug delivery applications for a few drugs [56, 40, 35].

This thesis aims at investigating preparation and characterization of porous and nonporous chitosan citrate membranes for transdermal drug delivery. The membranes were prepared by solvent casting method. The effects of the type of solvent, the presence of a plasticizing component polyethylene glycol (PEG), or glycerol and a porogen (NaCl) on the properties were investigated. Chemical structure determination was carried out by FTIR spectroscopy. The films were tested for their solubility, swelling and drug release properites at physiological pH, 7.4. The membrane morphology was

tested by SEM analysis. The final stage was loading naproxen as a drug model and testing for drug release which was followed by uv-vis spectrophotometry.

1.1 Polymers in Drug Delivery Systems

The polymers started to be used in drug delivery systems in the last twenty years. There are many issues should be considered when these polymers are used for human body such as stability *in vivo*, purity, safety and tuneable mechanical properties, in addition to suitable balance of hydrophobicity and hydrophilicity, appropriate charges, biocompatibility and biodegradability. Polymers got their importance in drug delivery systems due to the need to control concentration of the drug *in vivo* during suitable time period, in addition to targeting specific sites in human body without side effects. Moreover, these systems can adjust to be effective in therapeutic level (Figure 1) [1].

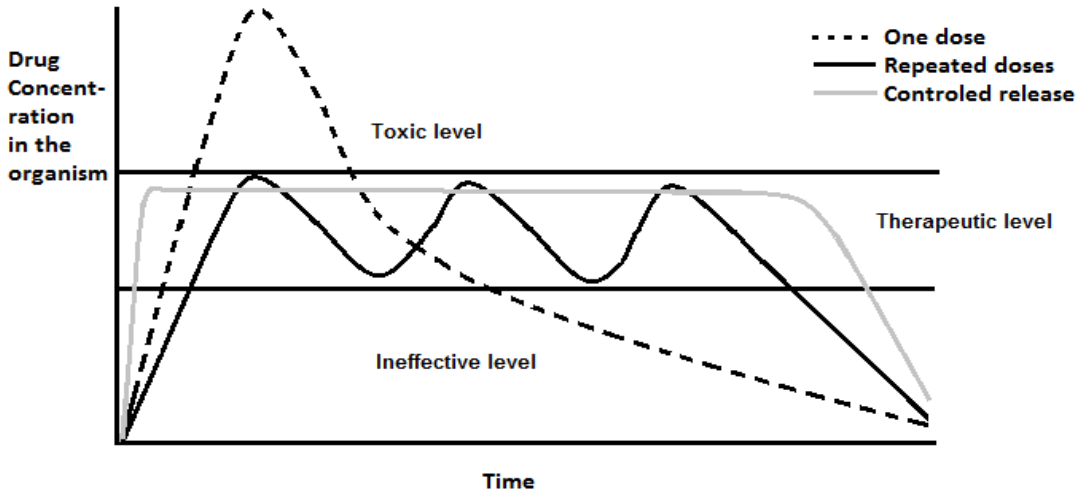


Figure 1: Effects of Drug Concentration by Four Administration Methods.

There are two ways of loading drugs into the polymers: physical and chemical loading. Physical methods can be achieved by matrix or reservoir systems. For the chemical method, it can be achieved by using ionic interaction or covalent linkage (Figure 2) [1].

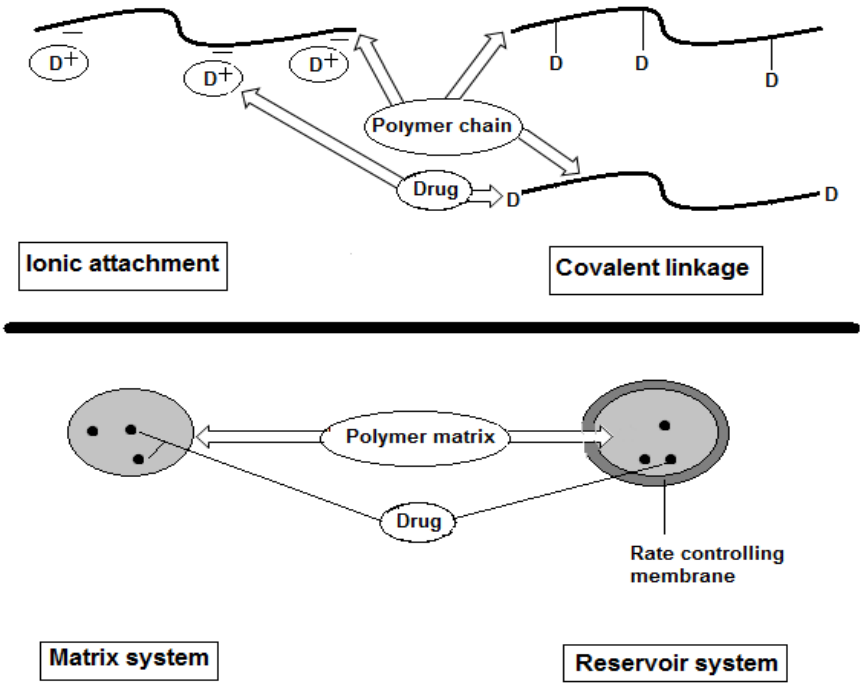
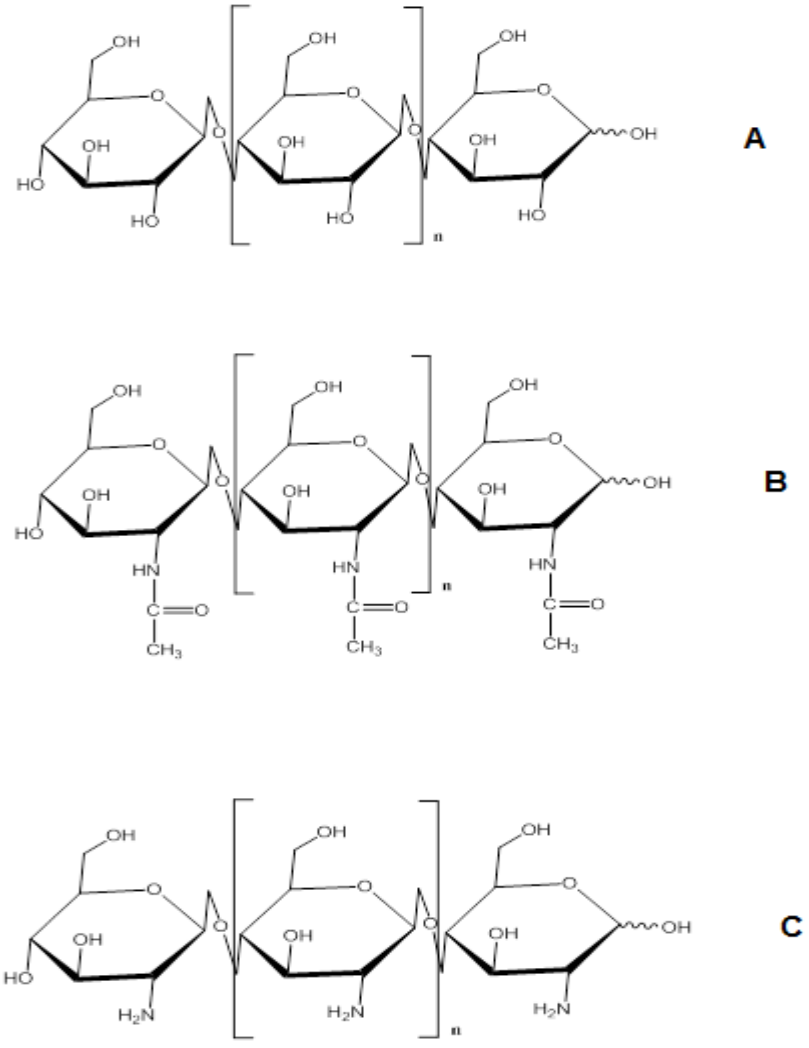


Figure 2: Four Types of Immobilization of Drugs into Polymeric system.

1.2 Chitosan and Chitin

Chitosan, poly[β-(1-4)-2-amino-2-deoxy-D-glucose] is classified as a natural polymer. It is an amino polysaccharide which can be obtained from chitin poly[β-(1-4)-2-acetamido-2-deoxy-D-glucose] after the deacetylation process. Chemically, the structure of chitin is similar to the structure of cellulose (scheme 1-A). The difference is that chitin has acetamide group instead of hydroxyl group at C-2 positions (scheme 1-B). Following the deacetylation process chitin acetamide group is replaced with amine groups, and this

replacement refers to the degree of deacetylation. This degree is a property utilized to distinguish chitosan from chitin chemically and terminologically. Chitosan (scheme 1-C) is considered as a linear copolymer of the above given two formulas. When the percentage of N- glucose amine is more than 50%, this polysaccharide is considered as chitosan, while if the percentage of N-acetyl glucose amine is more than 50% then polysaccharide called chitin.



Scheme 1: Chemical Structures of: A Cellulose, B Chitin and C Chitosan.

Chitin is the second most abundant polysaccharide after cellulose. Moreover, it is considered as the most abundant amino polysaccharide which can be obtained from natural sources. This natural polymer can be obtained from low sea animals or crustaceans such as shellfish, crabs and shrimps. Other sources to extract chitin are from oyster, jellyfish, algae, crayfish and fungi [2-4].

Historically, chitosan was discussed for the first time in 1859 by Rouget when he mentioned to the deacetylation processes for the essential parent of chitosan which is chitin. During the sixties of the last century, chitosan became subject of interest of many researchers. From that time, a huge amount of work on this natural polymer has been done and many papers have been published [5].

1.2.1 Properties and Applications of Chitosan and Chitin

Unique properties make chitosan and chitin get high attention in commercial, industrial and pharmaceutical aspects. An important chemical property is their high content of nitrogen when compared to other polysaccharides; Chitin and chitosan have 6.89% percentage of nitrogen. Most of the polysaccharides from natural origin are neutral or acidic in nature, while chitin and chitosan are basic polysaccharides; this gives them another range of differentiation as well. Furthermore, polyoxysalt and film formation, optical structural properties and chelation of metal ions are also considered as physical characteristics. Unlike cellulose which is a homopolymer, chitin and chitosan are considered as heteropolymers neither block nor random copolymers. Due to the solubility of chitosan in aqueous solution and the solubility of some of its derivatives in organic solvents makes chitosan superior to chitin which has poor solubility in these

solutions. This property is another attribute which makes chitosan suitable for both industrial and biological applications [2] [4-6].

After some modification processes on chitosan structure, it can be used for many non-biological applications, for example, food and nutrition processing, cosmetic, agriculture and waste water treatment. Many dangerous environmental problems solved by using chitosan to remove heavy metal ions and dyes from waste water. Due to its accumulation in the food chain, non-biodegradability and their effective on living species heavy metals are considered as one of the serious pollution in waste water. This problem can be solved by using chitosan gels, because of the ability of these gels to absorb heavy metal ions and dyes as well such as cadmium and chromophores respectively. Chitosan after treatment with certain acids can form a bridge between metal ions and these acids. The presence of amino groups within the structure of chitosan, which can introduce a positive charge in acidic media, exploits to absorb anionic dyes [7-9].

Many factors share out in the limitation of biological properties and activities for chitosan which are physiochemical factors, for example, molecular weight, crystallinity, the density of its charge in solution and degree of deacetylation. The important characteristics, like low cost, high biocompatibility, safety and biodegradability in vivo and *in-vitro*, open a wide range of advance medical and pharmaceutical application of chitosan. Anti-viral activity and inhibit the replication of some types of bacteria can be achieved by using chitosan derivatives as another application of this polymer which strongly depend on the above factors. Moreover mucoadhesive, swelling behaviour depending on pH changes, gel and film formation in addition to, micro/nano particles

forming, these characteristics make chitosan fits the purposes of the tissue engineering; wound healing and biological iron chelating. In addition to gene delivery, vaccine delivery and drug delivery systems are also applicable [5-6] [2, 10, 11].

As advance applications chitosan inter strongly to the field of nanoparticles in both environmental and biological aspects. For the environment it's reactive to absorb some dyes which cannot be removed by conventional methods. Yellow 145 which is a dye with harmful effects on the environment has been removed from industrial waste water by nanoparticles coated with chitosan. Chitosan based nanoparticles have been applied in nanobiotechnology for several purposes such as cancer diagnosis, targeting and imaging by using these nanoparticles [11,12].

From the above, it is important to know that all this wide range of applications of chitosan come from its unique properties and structure which have two active groups amino and hydroxyl. Many researchers try to exploit these properties of chitosan by doing some modifications. In order to obtain the fullest extent of benefit by improving mechanical properties and get more tunable behavior than using chitosan alone. Blending with other polymers or materials and introducing porosity are some examples that the researches have been reported. One of the most important modifications is the hydrogels formation which comes from chemical or physical interactions.

1.2.2 Chitosan Gels

Whether they're Gels or hydrogels from chitosan as a hydrophilic polymer, these gels are consider as three dimensional networks made up from crosslinking of chitosan. These gels are formed by covalent bonds (chemical crosslink) or by non-covalent bonds

such ionic, van der waals, hydrophobic interactions (physical crosslink) [13, 14]. Chitosan gels have many properties which give it a wide range of application for both biological and non-biological aspects.

1.2.2.1 Chitosan Chemical Hydrogels

These types of gels are considered as chemical gels, because it simply forms covalent chemical bonds between the polysaccharide and the crosslinking agent (Figure 3) [15].

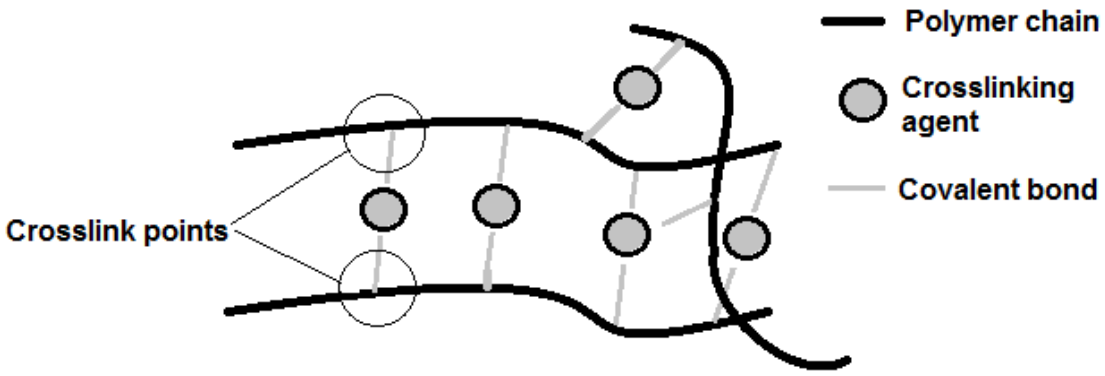


Figure 3: Chemical Covalent bond Gel

In addition to the main method of forming covalent chitosan gels by adding the cross linker to the chitosan solution, covalent gels can be formed by three secondary methods:

i. Chitosan chains cross-linked between each other without using crosslinking agent: this type form by crosslinking between two structural units of same and/or different chain of chitosan.

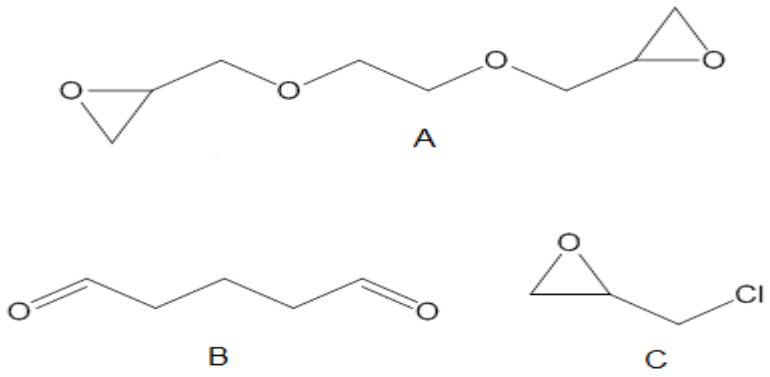
ii. Semi-interpenetrating polymer network (semi-IPN): this interpenetrating occurs by adding non-reacted polymer such as hemicellulose to the solution of chitosan, after that apply crossinking process. This will lead to entrap the non-reacted polymer into the crosslinked chitosan network.

iii. Full-interpenetrating polymer network (IPN): this is same as semi-interpenetration polymer, but the additional polymer is crosslinked as well. This leads to form two crosslinked networks, which in turn leads to the emergence of new characteristics.

iv. Finally, the fourth type is called hybrid polymer network (H.P.N.): this type of covalent gel obtained when the crosslinking occurs between chitosan chain units with another polymer chain unit, with the survival of the possibility of overlap between the same polymer chains with each other.

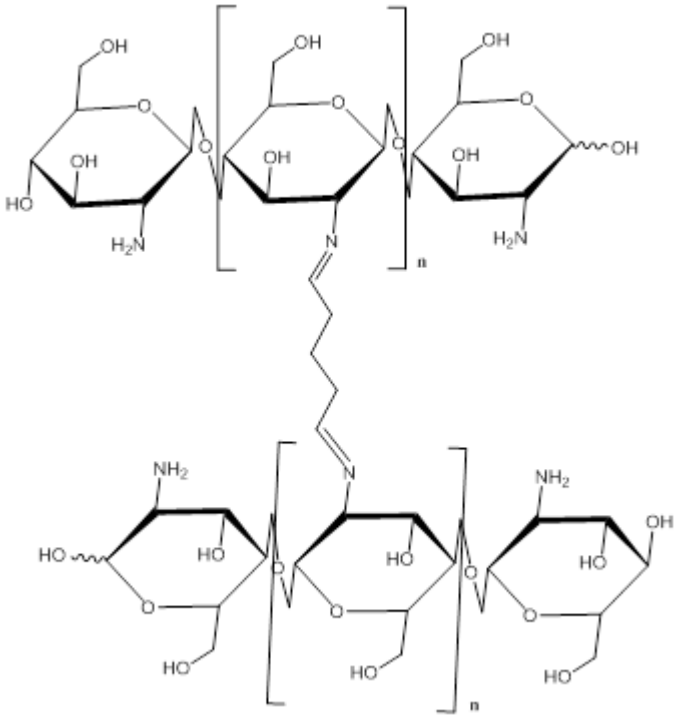
All the types of covalent gel with different crosslinking agent consider as porous gels. This property gave these gels a potential characteristic to use in many application needs this property especially in drug delivery systems [15, 16].

Due to it covalently bonds to form crosslinking network, covalent gels can also called chemical gels. Covalent gels considered as heterogeneous compounds, but it can be obtained in both homo- and heterogeneous conditions. By using poly-functional agent as a crosslinker these gels can be obtained. For chemical crosslinking purpose three crosslinkers will be mentioned due to it is wide range of applications, which are: EGDE, glutaraldehyde and epichlorohydrin (scheme 2) [17].



Scheme 2: Chemical Structures of: A- Ethylene glycol diglycidyl ether (EGDE), B- Glutaraldehyde (GA) and C- Epichlorohydrin

Dialdehydes are considered as the most popular chemical crosslinkers for chitosan. And the particular crosslinker between these dialdehyde is glutaraldehyde. These reactions occur covalently between aldehyde group of glutaraldehyde and amino group of chitosan (scheme 3). There are no linkage bonds forms with hydroxyl group of chitosan in this gel. Using glutaraldehyde as crosslinker has advantages, because of the possibility of using in aqueous media as direct reaction with no need to use catalyst. About the disadvantage, dialdehydes are believed to be carcinogenic compounds [15].

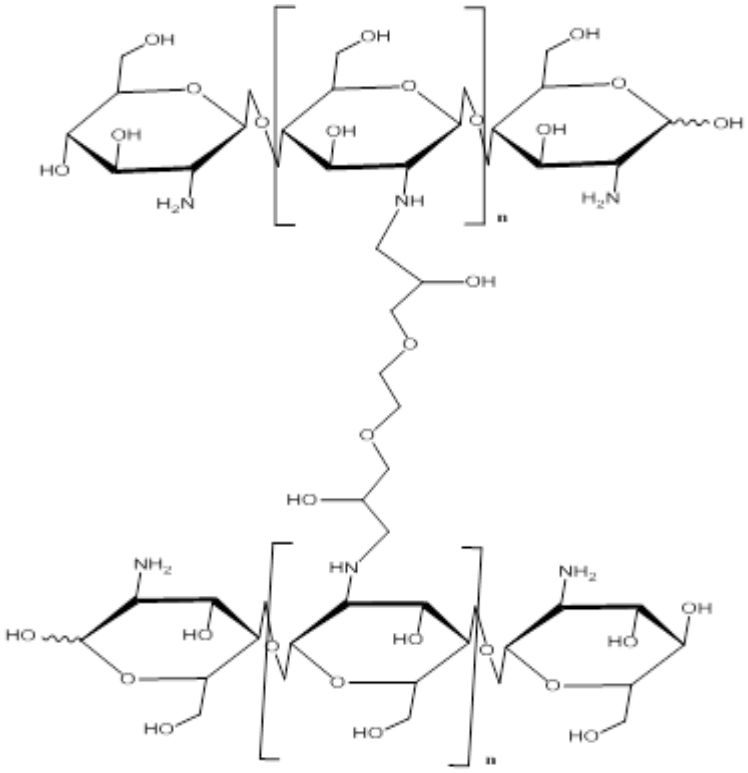


Scheme 3: Chitosan Crosslinked Glutaraldehyde

Like the other types of chitosan gels, many factors are taken into consideration in chitosan crosslinked glutaraldehyde. For example, pH value, temperature, degree of crosslinking and ionic strength. [18]. In addition to use glutaraldehyde with chitosan hydrogels basis, it is possible to use it with chitosan granules, microcapsules and fibers as well [19].

Chitosan is soluble in acidic media specifically gastric fluid, while chitosan crosslinked by glutaraldehyde can form microspheres used in oral drug delivery [oral delivery.]. For the non-biological application of chitosan crosslinked, glutaraldehyde gel can be used to remove heavy metals like lead(II) from polluted water [15, 21].

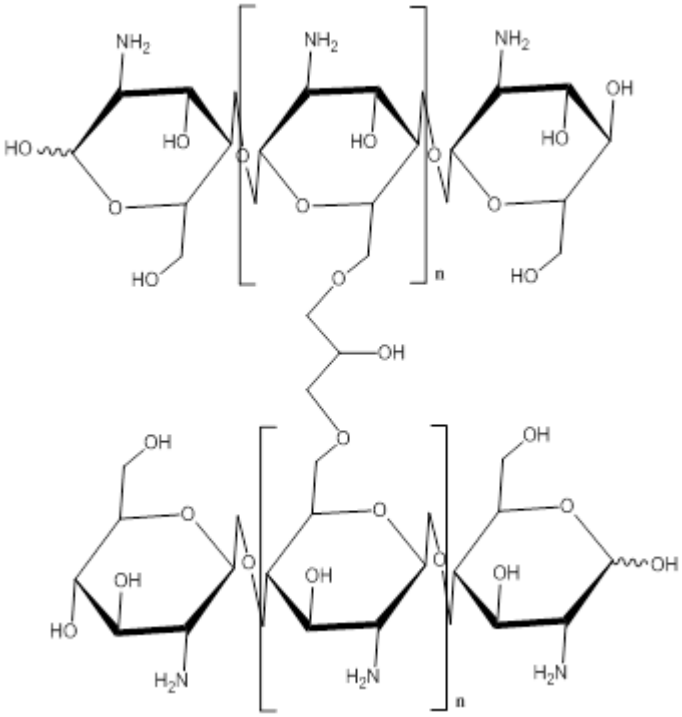
EGDE is another widely used crosslinker for chitosan (scheme 4). This gel can form semi-interpenetrating networks and give tunable swelling behavior with pH changes. Uncrsslinked polymer gels are subject to dissolve in solvents and swelling conditions. Therefore EGDE was used as a crosslinker to overcome this problem which can shows a good swelling behavior in any solvent without dissolve. Due to its high tunable swelling these gels have been used in drug release systems [22, 23].



Scheme 4: Chitosan Crosslinked EGDE

Epichlorohydrin is another common chemical crosslinking agent suitable to form chitosan gels for waste water treatment. In Scheme 5, crosslinking via hydroxyl groups is shown although it is highly probable that the amine groups also take part in the reaction unless protected. Depending on pH changes, temperature and concentration of

metal, this gel can be used as a good metal removal from waste water and industrial waste water.



Scheme 5: Chitosan Crosslinked Epichlorohydrin

According to studies of the properties of this gel it has a significant adsorption for Chromium (VI), Nickel (II) and cadmium (II) which cause environmental pollution and have high toxicity for the human, animal and plant. This removal occurs by adsorption/ chelating mechanism. Due to maintaining of the positive charge of chitosan and the negative charge of biological cell wall this gel has biomedical application as cell adhesion. Finally, this gel as the other types of chitosan gel has swelling and shrinking behavior so it can also use for drug delivery system [17] [24-27].

1.2.2.2 Chitosan Physical Hydrogels

Physical crosslinking gels have one advantage over the chemical crosslinkers; less toxicity. Many types of hydrogels can be obtained from physical interactions by using different carboxylic acids and these acids should be multi-functional groups to achieve three dimensional interactions between chitosan chains (Figure 4).

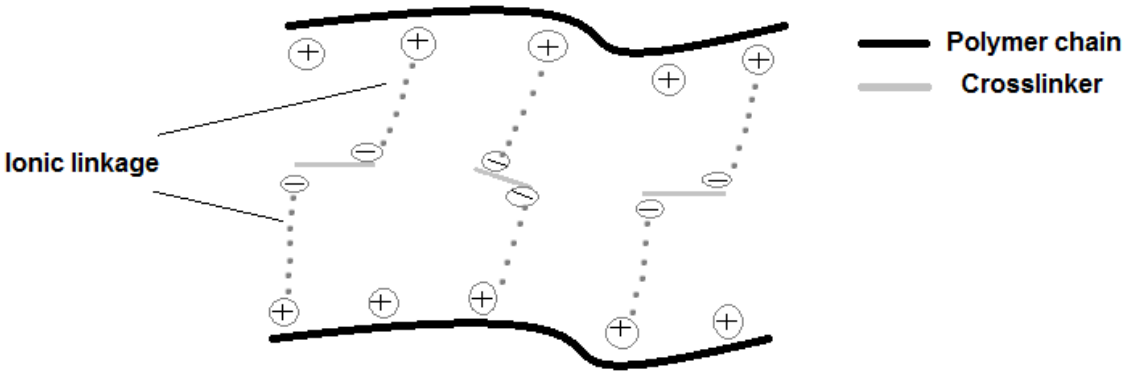
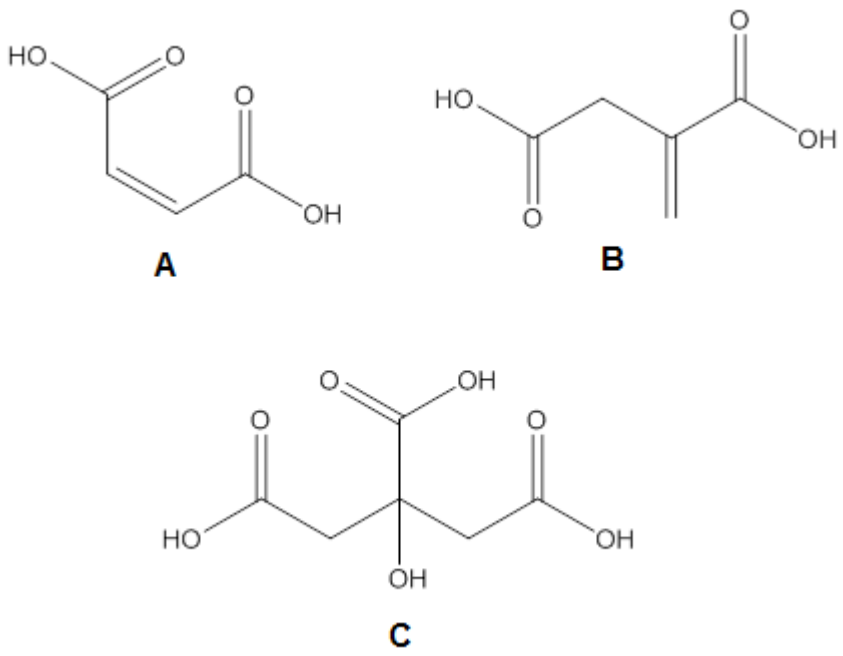


Figure 4: Physical Crosslinking (ionic) gel

Three types of physical crosslinked chitosan gels will be mentioned in this section, which are chitosan crosslinked by maleic, itaconic and citric acid as a crosslinkers (Scheme 6). The presence of multi carboxyl groups provides binding two or more chains of chitosan (depending on the number of carboxyl group). Moreover the biocompatibility and safety of these acids is important properties. And each of them gives different properties of the hydrogels because of the difference between the chemical structures of these carboxylic acids [13] [28-30].



Scheme 6: Chemical Structures of: A Maleic acid, B Itaconic acid and C Citric acid

The hydrogels of chitosan are obtained by dissolving it in the acid given of the above. The (COO^-) groups of these acids interact with (NH_3^+) group of dissolved chitosan and this form ionic cross linking between chitosan chains [14].

Due to its three dimensional structure, type of interactions and hydrophilic properties of chitosan make this hydrogels porous and sensitive toward pH changes that leads to swelling behavior in aqueous solution with different pH without dissolution or loss in the three dimensional structure [14].

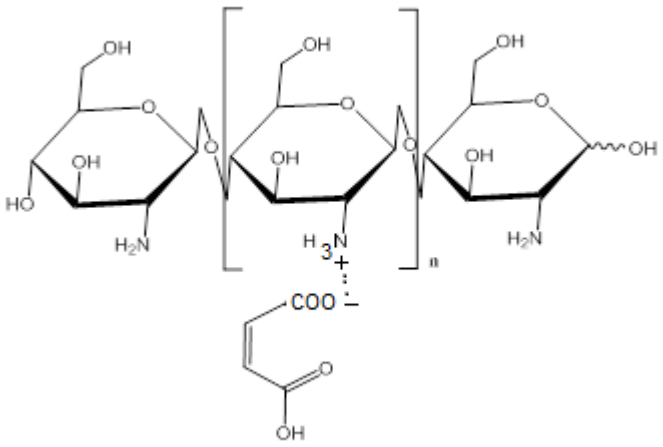
The swelling behavior is an important characteristic' of hydrogels and it depends on the crosslinking degree and the medium that surround the gel (pH and temperature). The main idea of this swelling behavior on one hand by repulsion forces between the positive

charges of chitosan ($NH3^{+}$) under acidic conditions, on the other hand by the repulsion force of the negative charge of the carboxylic acids (COO^{-}) under basic conditions. The change in pH leads to increase the positivity or the negativity of these functional groups, and in the two cases acidic and basic condition lead to change the osmotic pressure inside the gel, because of that we can get a different swelling ratio with different pH of the same gel [13, 14, 31]. At a given pH value, positive and negative charges on the gels form a complex leading to decrease in the swelling ratio. Hence, these are pH responsive gels.

This property gives it an important industrial application, for example agricultural, waste water treatment, treats environmental pollution by removing the heavy metals and separation of organic/water mixtures. On the other hand, wide ranges of medical applications are also possible [9, 13, 29] [32-34].

1.2.2.2.1 Chitosan/ Maleic Acid Gel

Physical gel can be formed by crosslink chitosan chains with Maleic acid (scheme 7), and this introduces a functional carboxyl and vinyl group of the maleic acid to the chitosan structure. This new structure facilitates in dissolution in water, since maleic acid decreases the crystallinity of chitosan due to the high branching degree when compared the gel of chitosan alone. Chitosan/Maleic acid gel is multi porous and has microgel morphology. In this gel, there are electrostatic, hydrophobic and H-bonding interactions. Because the presence of hydrogen bonding supplied through amino, amide, carboxyl and hydroxyl groups' solubility in water became available.



Scheme 7: Chitosan Crosslinked Maleic acid

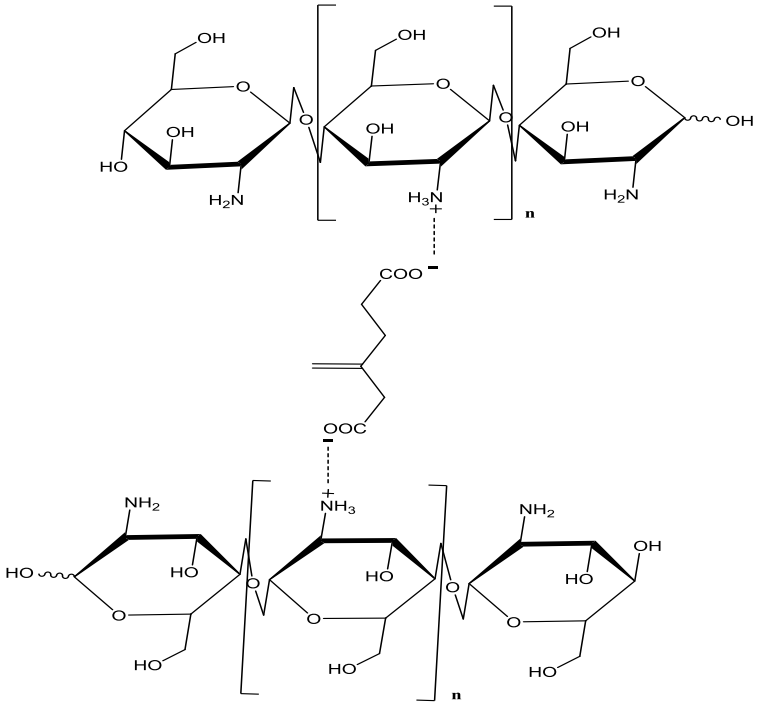
The great property in this gel that gives a wide range of medical applications is the safety and chain entanglements. This entanglements forms because, of hydrophobic moieties that represented by vinyl and acetyl groups. Another characteristic we can get from this gel is the significant increase in swelling if we compare it with the low swelling ratio of chitosan alone. The medical applications of this gel are tissue engineering, drug delivery, antimicrobial (negative germs). These applications are due to the improvement of the hydrophilicity (decrease in water contact angle), and introduce carboxyl group (change in protein adsorption, spreading and prolification). Also it improves cell adhesion and compatibility to promote fibroblast attachment, because the cell spread is good on chitosan/Maleic gel [9, 29].

Maleic acid has been used for surface modification for the already crosslinked chitosan with another crosslinker, for example glutaraldehyde as an advanced modification. This type of modification gives the gel high selectivity to the water due to the increase in the

hydrophilicty (water interactive) of the surface caused by introducing carboxyl group to the surface. This leads to enhance the pervaporation performance of this gel. High performance separation between the organic phase and the water phase can be achieved by using this type of gel, supplying another application for this gel [32].

1.2.2.2.2 Chitosan/ Itaconic Acid Gel

Itaconic acid has a broad use in hydrogel formation, because it forms high pH sensitive gel with amino group within chitosan. This happen due to COOH group in the itaconic ionized to COO- that gives itaconic acid its sensitivity toward pH changes. High swelling behavior also can be achieved depending on this sensitivity, in addition to the high porous property of this gel (scheme 8) [13, 14].



Scheme 8: Chitosan Crosslinked Itaconic acid

The essential feature of this hydrogel is that many substances can be add to this gel to get more advantages from it. For example, free radical polymerization by adding methacrylic acid can give this gel better mechanical properties and more tunable

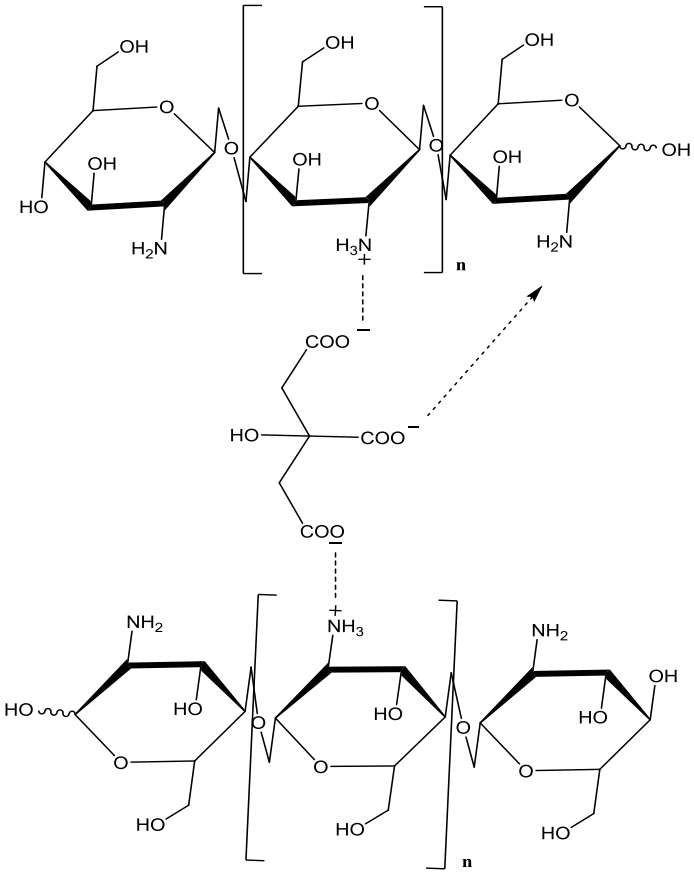
swelling. In addition, the swelling kinetics of this gel that can change by adding methacrylic acid which causes to increase the swelling ratio with increase in methacrylic acid specifically at low pH (positive charge repulsion). The degree of porosity of this modified gel can be controlled by the amount of methacrylic acid. This type of modification can be used in the field of drug delivery by high control drug release anticancer drugs or proteins, cell immobilization, carriers for genetic modification, autoduplication of DNA, and also for non-biological application such as the removal of the heavy material (that have high toxicity) from the waste water and from the polluted environment [13, 14, 34].

Another addition that can be introduced to this gel is poly(vinyl alcohol) which has great chemical and physical and biological properties such as the biocompatibility, water solubility, flexibility, chemical stability and safety. Poly(vinyl alcohol) can be added to the gel through side chain aggregation, and intermolecular and intramolecular H-bonds. We can gain all the properties of poly(vinyl alcohol) by adding it to chitosan/Itaconic gel. Moreover the pore size, hydrophilicity and the swelling ratio can be reduced due to the formation of the new covalent bonds. Potential application in term of control drug release in drug delivery system can be achieved via this type of addition [33].

The last addition example is the introduction polyacrylamide to the chitosan/Itaconic gel that gives swelling behavior in at pH 4, pH 10 and neutral medium. A representation example is its application for ascorbic acid (vitamin C) delivery [31].

1.2.2.2.3 Chitosan/ Citric Acid Gel

Citric acid has three carboxylic functional groups that give it high ability to be a good crosslinker for chitosan (scheme 9). Through exploit the attraction between the positive charges of the chitosan with the negative high density charge of the citric acid, a gel can be obtained by adding sodium citrate to chitosan solution, or soaking the already prepared chitosan film in sodium citrate solution.



Scheme 9: Chitosan Crosslinked Citrate

This gel has a distinctive surface morphology such that the bottom and upper are different, one is smooth and the other one is rough respectively. This property has it is significant effect on its application which represented by drug delivery in terms of the

size of pores and degree of roughness [13]. Coating technique by poly anion (due to high negative density of citric acid) is used for this type of gel in order to prolong drug releasing period. In addition to high swelling ratio, safety and film forming of this gel properties makes this gel suitable for wound healing, delivering many types of non-polar drugs, such as riboflavin, indomethacin, 5-FU and Theophylline [1, 30, 35, 36].

1.2.3 Chitosan Based Membranes

Many formations in different scales can be obtained from the polymers such as microsphere, microcapsules, gels and membranes. The membrane form is widely used for transdermal delivery systems. Transdermal systems are considered as the most widely used form of drug applications which is friendly with the patient, because of it is softy and harmless to the skin pores. Following the delivery and penetration through the skin, passes-through many membranes reach to tissues. Many therapeutic applications of transdermal systems such as painkiller plaster, anti-nicotinic treatment and heart disease are available.

Chitosan as mentioned above can be converted to gels which have industrial, environmental and many biological applications. High tenable properties can be obtained through modifications on chitosan. For example, combining with other polymers, chitosan can be used for oral, mucosal, colonic and ophthalmic drug delivery. In addition to its biocompatible, biodegradable, and bioactive and safe properties, chitosan has a good membrane forming capacity.

Chitosan based membranes show bioadhesion, swelling, and good mechanical properties depending on pH. These characteristics make chitosan based films suitable for many pharmaceutical applications. Wound healing, antibacterial activity and drug delivery are examples for these applications. Using chitosan alone generates hydrogels and it is incompetent for controlled drug delivery [37, 38].

Chitosan requires different modification processes in order to fit various pharmaceuticals. Introducing or crosslinking with other polymers or addition of plasticizers are methods to enhance different properties for membranes for the purpose of drug delivery systems. For example it is possible to obtain a promising drug delivery system after crosslinking with poly(acrylic acid) through improving pH-sensitivity and that gives hydrogel available to deliver amoxicilline to specific sites. Adding glycerol as plasticizer to this hydrogel can produce membranes with non-rigid, swelling properties, but they are insoluble in water, less glass transition temperature and high pH swelling dependency. This membrane is promising for transdermal drug delivery systems [38].

Blending chitosan with hydroxypropyl methyl cellulose (HPMC) will give comfortable membrane due to it is transparency, good film forming and high mechanical properties. This membrane shows increasing mechanical performance with increase the content of HPMC. Anti-arrhythmic and anti-hypertensive drug, propranolol HCl, is a model drug that can be delivered by using transdermal delivery. This drug is efficiently diffuse by using chitosan/HPMC membrane. Addition of glycerol as plasticizer and sodium citrate for crosslinking is an advance modification for chitosan/HPMC membrane. This

membrane can be used as transdermal delivery system for anti-inflammatory etoricoxib [39, 36].

In addition to the transdermal drug delivery applications, chitosan membranes can also be useful to simulate skin permeability. By forming an artificial membrane, modified chitosan membranes can be used to stimulate the skin for mimic the permeation of drugs through skin. A representation example is a biomimetic chitosan membrane combined with N-arginine. This system can be used for adefovir transdermal delivery. As an advance using for chitosan membranes it can also be manufactured for nano-scale applications as seen for amphoteric multilayer thin film. This film can be useful for potential nanotechnology applications [40, 41, 22].

1.2.3.1 Chitosan Porous Membranes

Good membranes formation as well as the other properties of chitosan, thus, make chitosan suitable for transdermal drug delivery and wound dressing which is mentioned extensively detail in the aforementioned sections. In many situations of the above applications, porous membrane structures are the eligible ones. For introducing porosity, the researchers have developed several procedures; including, freeze drying, high pressure gasification and porogenic agents' methods. Two types of macro-porous chitosan membrane can be obtained in symmetric and asymmetric porous membrane structure. Symmetric structure can be produced by using porogen agents. The membrane which was obtained from this method gives production of high specification yield. Another good feature of porous membrane which obtained after treating with porogen agent is that it shows possibility of controlling the rate of pore size easily [42, 46].

1.2.3.1.1 Porosity by Using Porogen Agents Technique

Practically, the procedure of using porogen agent for introducing porosity can be summarized by: dissolving the porogen agent in the chitosan solution, followed by evaporating the solvent and washing the product to remove the porogen agent. The method of utilization of silica or sodium chloride as porogen agents for chitosan to produce symmetric porous membranes has been reported. For the application of such membranes the researchers stated that it can be used for adsorption of bacterial toxins, human proteins such as albumin and concanavalin. Porogen/chitosan membranes have essential flaws because it has low mechanical properties and low selectivity. To eliminate the issue of mechanical properties, these membranes should be crosslinked by using crosslinking agents.

Formation of the other macro-porous membrane which is asymmetric type, it can overcome all symmetric type drawbacks. Asymmetric porous membranes have special properties such as thick structure layer and sponge sub layer which make it preferably for wound dressing applications. For producing this type of chitosan membranes chitosan solution casted and then drown in to coagulation bath for long time. After that, chitosan solution is separated in to two phases, polymer rich and polymer poor contain. Different structures can be formed by controlling separation operations like temperature and time of evaporation. Asymmetric membranes have well selectivity and high flow rate. By combining the two methods of producing the membrane symmetric and asymmetric to produce a membrane has the advantages of both. This membrane can be used for specific drug models such as sodium sulfamerazine and sulfametoxipyridazine [42].

Since silica is high cost material and not easy to remove, some researchers prefer to use sodium chloride as a porogen agent for preparing chitosan porous membranes. The researchers prepared a chitosan-silane hybrid membrane, and introduced porosity depending on sodium chloride porogen agent. The membrane was crosslinked by GPTMS (glycidoxypropyltrimethoxysilane) as crosslinking agent. Formed membrane possesses an anisotropic macroporous structure, with microporous structure within the skeleton of the macroporous main morphology. This hybrid porous membrane is used for affinity sorption, enzyme immobilization, fuel cells and tissue engineering [43].

Using calcium carbonate, also as porogen agent, is another method to form porous membrane. Calcium carbonate is trapped inside the structure of the chitosan, followed by acid decomposition and changing the temperature to form porous membrane. The researchers prepared a hybrid membrane between chitosan as a natural polymer and polydimethylsiloxane as inorganic polymer. They found that the well mechanical properties of chitosan membranes were not affected through this chemical modification. In addition to that, the produced membrane has higher decomposition temperature compared to the pure chitosan membranes in thermogravimetric analysis [44].

1.2.3.1.2 Porosity by Using Freeze-Gelation Technique

After doing ionic crosslinking for chitosan on one hand, followed by covalent crosslinking on the other hand, freeze-gelation method has also been used in for introducing porosity. Sulfate used as ionic crosslinker and EDC/NHS agent used as covalent crosslinker. Freeze-gelation method can form a highly porous membrane. Changing the weight ratio of chitosan and sulfate gives diversity of the characteristics of these membranes. The hydrophobic behaviour of chitosan cause low cell attachment

and therefore less range of biomedical application. This behaviour will be cancelled after doing the above modification processes. The resulting membrane has high tensile strength, hydrophilic and it is biocompatible and biodegradable. Therefore it has good properties for utilizing as biomaterial for biomedical application [45].

1.2.3.1.3 Porosity by Using Freeze-Drying Technique

Recently, freeze drying for introducing porosity is successfully used for introducing porosity for chitosan membranes. The researchers have prepared several porous chitosan membranes using this technique. One of the examples for freeze drying method was making crosslinking chitosan with alginate (as antibacterial active natural polymer) when calcium ions were used as crosslinking agent that generated no porosity. The preparation followed by freeze drying method introduces porosity. The procedure of membranes can be useful for biomedical applications and produce highly porous scaffolds promising for tissue engineering. Freeze drying method has some limitations, such as, high cost, difficult up-scalability and long period to make [46, 47].

1.2.3.1.4 Other Techniques

As another technique, following the bending chitosan with alginate, the porosity was introduced using pluronic F68 and tween 80. Using alginate gives these membranes an antibacterial property, thus it can be utilized for wound dressing. The produced membranes have good mechanical properties and high porosity so it can be used in tissue engineering field as scaffolds [46, 47].

Introducing sustainable supercritical CO_2 to chitosan with inversion processes induced phase technique; it is also a method for getting chitosan porous membrane. Simply, this technique based on steaming the CO_2 during induced process that introduces porosity for

the formed membrane. This membrane is suitable for drug delivery, cell adhesion, tissue engineering and regenerative medicine [48].

Production of porous chitosan membranes received attention from researchers for several biomedical and pharmaceutical applications. Many methods were tried to obtain chitosan films and introducing porosity to these films. A thermo sensitive chitosan hydrogel can be obtained after treating chitosan solution with glycerophosphate at 37° C temperature. Methodologically, chitosan solution is neutralized using glycerophosphate that forms a macroporous hydrogel having thermo sensitive properties. The macroporous membrane can be obtained after casting and then doing an evaporating process to this hydrogel. This membrane has a high porosity, safety, biocompatibility and high protein adsorption properties. Chitosan porous membrane based on chitosan/glycerophosphate hydrogels can be utilized for drug-, enzyme-, and protein-delivery and also in tissue engineering field [49].

Using chitosan alone in highly concentrated solution can give porous hollow fiber membrane was also used. A concentration of chitosan reached to 18 % weight chitosan in acetic acid solution. The forming membrane was hollow fiber structure. It was observed that the mechanical properties of this membrane were high in the wet state. This membrane can be used for filtration and adsorption processes. The main application for this type of membrane is for waste water treatment especially for copper ions adsorption [50].

Finally, the porosity is not just introduced by many methods; it is also introduced and investigated by several scales. Nano porous chitosan membranes are achieved by

advanced several ways, such as, electrodeposition method. A third dimensional nano-porous chitosan membrane was formed by this method. The formation membrane can be used in biosensing and biomolecule immobilization. Using porogen agent for reaching to nano scale is also another method to introduce porosity, and getting carbon nano tubes within the structure of chitosan. The produced membrane can be used for pharmaceutical applications such as wound dressing, and for industrial applications as well such as water treatment [51, 52].

1.2.3.2 Chitosan Citrate Membranes

It is known that the drug delivery process required modifications and additions to chitosan to reach the purpose required. These processes are in order to fit the type of drug in terms of oral, nasal, transdermal and wound healing, on the other hand in terms of control of the rate of drug release per hour. Improving mechanical properties is also important that requires modification. One of the important improvements on characteristics of chitosan includes using chemical cross-linking methods such as a chitosan cross-linked with glutaraldehyde, acetate, pectin, xanthan, tripolyphosphate or citrate which is the subject in this research. Using of carboxylic acids with multi carboxyl groups (like citric acid), is acceptable pharmaceutically. In this study, chitosan membranes produced through many chemical and physical modifications processes, including the crosslinking using sodium citrate to obtain new properties, were examined for some of the pharmaceutical purposes described above. In addition to the advantages of chitosan which mentioned above, the choice of sodium citrate as has important considerations. Sodium citrate is the salt of citric acid, which is involve in Kreb cycle and used as food additive and water softening in our daily lives [13, 53, 54].

As referred above, modification through crosslinking agents is one of the most important processes which effect mainly on the characteristics of chitosan membranes. Thus the selection of the crosslinker is important to determine and obtain the highest properties of these membranes. As an important factor, the effect of water on chitosan membrane is deeply changed after crosslinking by citrate [55].

Chitosan/citrate films are produced through exploiting the advantages of electrostatic interactions between citrate and chitosan (this explains the use of non-polar drugs with this type of delivery systems). This cross-linked happens by steeping the chitosan membranes in different concentrations of aqueous solution of sodium citrate and for certain cross linking times the cross linking between chitosan(poly-anion) and citrate(poly-cation) makes these membranes have degree of ionization allow to it swelled when pH changes. The pores of these films that the drug loaded in it will be wider after swelling, that make the drug release out of these films in a different rate depend on the size of this pores and consequently depends on pH; this status gives the films its effectively characteristics of drug delivery systems. In addition of the impact of pH on chitosan/citrate films, there are several factors, such as molecular weight of the chitosan which has a significant impact on the properties of chitosan and degree of deacetylation; moreover some other factors such as parameters in film preparation, the salt concentration in media, polyanion coating, model drug nature also effect on film swelling and drug release from this films.

Representative drugs delivered through chitosan cross linked citrate are non-polar drugs (e.g. riboflavin, indomethacin, 5-FU and theophylline). All of these drugs and other have

a different routes and conditions for loading and releasing from chitosan citrate membranes [13, 14].

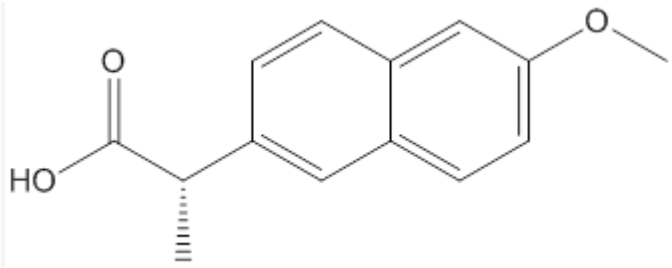
Chitosan citrate membranes compared with chitosan acetate previously. This study showed that these membranes can be used for wound healing purposes, but chitosan citrate membranes shows better performance. In this study the porosity can be introduced to chitosan citrate membrane by using freeze-drying technique, and the porous size can be controlled by varying this temperature [35].

The investigation on chitosan citrate membranes showed that chitosan citrate is hydrocolloidal matrix and stable membrane. For the drug release studies, it is possible to colour chitosan citrate membrane with dyes. Common dyes that might be used for colouring chitosan citrate membranes include brilliant blue, sunset yellow, green FS and many others. These dyes have been used for investigating the release of a model of drug propranolol HCl using FTIR and DSC spectroscopic analyses. Chitosan citrate membrane shows satisfactory result in it is properties for the pharmaceutical uses [56].

Not just as membranes for transdermal delivery and wound healing, chitosan crosslinked which citrate can form microspheres for nasal and oral delivery, and as a gel for vaginal drug delivery. For the nasal delivery the excellent mucoadhesive property makes this microspheres suitable for prolong the release of salbutamol to a period reach to 24 hour [57, 58].

1.3 Naproxen as Drug Model

Chemically, naproxen considers as a type of propionic acid, it has the formula $C_{14}H_{14}O_3$, according to IUPAC nomenclature naproxen called (S)-2-(6-methoxy naphthalene-2-yl) propanoic acid (Scheme 10).



Scheme 10: Chemical Structure of Naproxen

Biochemically, naproxen can inhibit cyclooxygenase-1 and cyclooxygenase-2, and subsequently it prevents prostaglandin synthesis. High plasma protein binding is another property of naproxen due to it is functional groups. Therapeutically, naproxen is an anti-inflammatory agent, widely used for inflammatory disorders. Naproxen is a poor soluble compound, thus loading naproxen into chitosan membrane can enhance its dissolution rate and accelerate its analgesic effect. By utilizing this and the other properties of chitosan which mentioned in the above sections, chitosan can be used for design transdermal drug delivery system for naproxen. Hepatic and metabolism negative effects of this drug can be diminish by using the rout of transdermal delivery. [59, 60]

Naproxen can be also combined with chitosan to produce microparticles for oral delivery system. This will lead to reduce the harm of the domestic GIT mucosal due to the wide distribution of the membrane microparticles over a vast area, compared with homogeneous drug dosage. Brain delivery is another approach to deliver naproxen using

various chemical delivery systems. Using copolymer contain chitosan is an advance to design delivery system for naproxen in nanoscale techniques to prepare magnetic nanoparticles loaded with naproxen [61-64].

Chapter 2

EXPERIMENTAL

2.1 Materials

The materials that have been used in this work are shown in Table 1. They were all used as received. Double distilled water used for preparing the solutions was obtained in our laboratory.

Table 1: Materials and it is Manufacturing Company.

Material	Manufacture
Chitosan medium molecular weight practically obtained from carb shells	Aldrich, Germany
Acetic acid	Sigma-Aldrich, Germany
Sodium Citrate Tribasic Dihydrate	Sigma-Aldrich, Germany
Sodium hydroxide	Sigma-Aldrich, Germany
Sodium Chloride	The British Drug House, UK
Potassium dihydrogen phosphate	Sigma-Aldrich, Germany
Poly(Ethylene Glycol), average MN CA. 8000 (PEG 8000)	Sigma-Aldrich, Germany
Glycerol	Sigma-Aldrich, Germany
Naproxen	PharmaModial, North Cyprus

2.2 Solution Preparation

2.2.1 Preparation of Citrate Solution

This solution was prepared by dissolving 12.5 g of trisodium citrate powder in 500 mL water and kept at room temperature.

2.2.2 Preparation of Phosphate Buffer Solution

Phosphate buffer solution (pH 7.4) was prepared by mixing (0.1 M) 100 mL of potassium dihydrogen phosphate solution with (0.1 M) 78.2 mL of sodium hydroxide, and dilution till 200 mL. The pH of the buffer is preferentially chosen to simulate to the physiological pH.

2.2.3 Preparation of Acetic acid solution

This solution was prepared by diluting 10 mL of 99.9 pure concentrated acetic acid solution by double distilled water in volumetric flask (total 1000 mL). This gave 1% (v/v) acetic solution used after that for the purpose of dissolving chitosan.

2.2.4 Preparation of Chitosan Solutions

0.5 g or 1.0 g of chitosan was dissolved in 100 mL of 1% (v/v) acetic acid under stirring at 500 rpm overnight to prepare 0.5% w/v or 1% w/v chitosan solution respectively.

2.2.5 Preparation of Sodium Chloride Solution

Sodium chloride solution was prepared by dissolving 23.3 g of NaCl in 100 mL double distilled water.

2.2.6 Preparation of 1 M of sodium hydroxide solution

This solution was prepared by dissolving 20 g of NaOH in 500 mL double distilled water. This solution was prepared for the purpose of removing the membrane from the petri dish after drying.

2.3 Membrane Preparation

2.3.1 Sample 1

0.5% chitosan solution was filtered. 25 mL of this solution was poured into a glass petri dish and dried in the oven at 45°C for 24 h to cast membrane. Then, dried film was removed from the petri dish with 25 mL of 1 M NaOH solution, washed with double distilled water and was soaked into 2.5% trisodium citrate solution at 4°C for 48 h for ionic crosslinking. The sample was dried at room temperature.

2.3.2 Sample 2

0.5 % chitosan solution was filtered. 25 mL of this solution was poured into glass petri dish and dried in oven at 45°C for 24 h to cast the membrane. Then dried film was removed from petri dish with 25 mL of 1 M of NaOH solution. The membrane formed had been washed with double distilled water then was soaked into 23.3% NaCl solution at 4°C for 48 h. Then films were washed with double distilled water and were soaked into 2.5% trisodium citrate solution 4°C for 48 h for ionic crosslinking and dried at room temperature.

2.3.3 Sample 3

0.5% of chitosan was filtered. Then, 0.0625 g of PEG (8000) was added into 100 mL of prepared chitosan solution and stirred 8 rpm/1h. 25 mL of this solution was poured into glass petri dish and then dried in the oven at 45°C for 24 h. Then, the dried film was

removed with 1 M 25 mL of NaOH. After that it was washed with double distilled water and was soaked into 2.5% trisodium citrate solution at 4°C for 48 h for ionic crosslinking and dried at room temperature.

2.3.4 Sample 4

0.5 % of chitosan solution was filtered. Then, 0.0625 g of PEG (8000) was added into 100 mL of prepared chitosan solution and stirred 8 rpm/1h. 25 mL of this solution was poured into glass petri dish and then dried in the oven at 45°C for 24 h. Then, the dried film was removed with 1 M 25 mL of NaOH. The formed film was soaked into 23.3% NaCl solution at 4°C for 48 h. After that, the produced membrane has been washed with double distilled water. Finally, was soaked into 2.5% trisodium citrate solution 4°C for 48 h for ionic crosslinking before dried at room temperature.

2.3.5 Sample 5

0.5 % chitosan solution was filtered. Then, 0.125 g of PEG (8000) was added into 100 mL of prepared chitosan solution and stirred 8 rpm/1h. 25 mL of this solution was poured into glass petri dish and then dried in the oven at 45°C for 24 h. Then, the dried films were removed with 1 M 25 mL of NaOH. The membrane had been washed with double distilled water. Finally, were soaked into 2.5% trisodium citrate solution 4°C for 48 h for ionic crosslinking and dried at room temperature.

2.3.6 Sample 6

0.5 % chitosan solution was filtered. Then, 0.125 g of PEG (8000) was added into 100 mL prepared chitosan solution and stirred 8 rpm for 1 h. 25 mL of this solution was poured into glass petri dish and then dried in the oven at 45°C for 24 h. Then, the dried film was removed with 1 M 25 mL of NaOH. The formed film was soaked into 23.3%

NaCl solution at 4°C for 48 h. Then the membrane washed with double distilled water. Finally, was soaked into 2.5% trisodium citrate solution 4°C for 48 h for ionic crosslinking and dried at room temperature.

2.3.7 Sample 7

1 % Chitosan solution was filtered. Then, 23.3 g of NaCl was added into 100 mL of prepared chitosan solution and stirred 8 rpm till homogenized. 25 mL of this solution was poured into glass petri dish and then dried in the oven at 45°C for 24 h. After drying, the film was washed with excess double distilled water till remove all NaCl particles. Then, it was dried again at 45 °C till complete drying. Then, dried film was removed with 1 M 25 mL of NaOH. Then membrane washed with double distilled water and finally was soaked into 2.5% trisodium citrate solution 4°C for 48 h for ionic crosslinking before dried at room temperature.

2.3.8 Sample 8

1 % Chitosan solution was filtered. Then, 23.3 g of NaCl was added into prepared chitosan solution. After that 10 g of glycerol was added into prepared solution and stirred at 8 rpm till homogenized. 25 mL of this solution was poured into glass petri dish and then dried in the oven at 45°C for 24 h. After drying, the films were washed with excess double distilled water till remove all NaCl particles. Then, it was dried again at 45 °C till complete drying. Then, dried films were removed with 1 M 25 mL of NaOH. They were washed with double distilled water and finally were soaked into 2.5% trisodium citrate solution 4°C for 48 h for ionic crosslinking and dried at room temperature.

2.3.9 Sample 9

1 % chitosan solution was filtered. 25 mL of this solution was poured into glass petri dish and then dried in the oven at 45°C for 24 h. Then, the dried films were removed with 1 M 25 mL of NaOH. Then washed with double distilled water and were soaked into 2.5% trisodium citrate solution 4°C for 48 h for ionic crosslinking and dried at room temperature.

2.3.10 Sample 10

1 % chitosan solution was filtered 25 mL of this solution was poured into glass petri dish and then dried in the oven at 45°C for 24 h. Then, dried films were removed with 1 M 25 mL of NaOH. The formed film was soaked into 23.3% NaCl solution at 4°C for 48 hours. Then washed with double distilled water and were soaked into 2.5% trisodium citrate solution 4°C for 48 h for ionic crosslinking and dried at room temperature.

A summary of preparation conditions are given below.

Sample	
1	0.5% chitosan/ crosslinked (blank sample)
2	0.5% chitosan soaked in NaCl solution/crosslinked
3	0.5% chitosan + 0.0625 g of PEG/ crosslinked
4	0.5% chitosan + 0.0625 g of PEG soaked in NaCl solution/ crosslinked
5	0.5% chitosan + 0.125 g of PEG/ crosslinked
6	0.5% chitosan + 0.125 g of PEG soaked in NaCl solution crosslinked
7	1% chitosan Treated with NaCl/ crosslinked
8	1% chitosan Treated with NaCl + PEG/ crosslinked
9	1% chitosan/ crosslinked (blank sample)
10	1% chitosan Soaked in NaCl solution/crosslinked

2.4 Swelling Test

Swelling kinetics were followed by weighing a small piece approximately 1x1 cm^2 from each membrane by electronic balance. Then each piece was soaked separately in 15 mL double distilled water. Each piece had been blotted with filter paper to remove the water from the surface, and then weighed again after 0.25h, 0.5h, 1h, 2h, 3h, 4h, 5h, 6h, 18h and 42h respectively.

2.5 Drug Release Experiment

This experiment is based on three elements. First; plotting a calibration curve for the model drug which is naproxen, second; loading the drug into the membranes and third;

finding the release profile for each membrane. Drug release was followed by using T 80 + UV\VIS Spectrometer PG Instrument Ltd.

2.5.1 Naproxen Calibration Curve

Beer's-Lambert calibration curve for naproxen was obtained by preparing a series of different concentrations of naproxen. The solvent was used for dissolving naproxen and for the dilution was phosphate buffer solution pH 7.4. The concentrations of naproxen were: ($5*10^{-5}$), ($6*10^{-5}$), ($7.5*10^{-5}$), ($8*10^{-5}$), ($9*10^{-5}$) and ($10*10^{-5}$). The absorbances of these concentrations were measured using uv-visible spectrophotometer set at 230nm wavelength.

2.5.2 Entrapping of Naproxen (Drug Loading)

For the purpose of naproxen loading into the membranes and release of naproxen from the membranes, six membranes had been chosen among the above given ten membranes. They were sample 1, sample 3, sample 4, sample 7, sample 8 and sample 9. The samples were cut into 1*1 cm^2 pieces in size to conduct the experiment. Naproxen solution was prepared by dissolving naproxen (0.0156 g) in 100 mL phosphate buffer solution of pH 7.4 (the blood pH). Each membrane was soaked separately in 25 mL of this solution for 30 minutes. The amount of naproxen, which was loaded into each membrane, had been determined gravimetrically.

2.5.3 *In-vitro* Release Study (Drug Release)

In this experiment the samples 1, 3, 4, 7, 8, and 9 (loaded with naproxen) were put separately in one beaker containing 20 mL of phosphate buffer solution of pH 7.4. These beakers were placed in a water bath of 37°C under stirring at 100 rpm. After that 3 mL

was withdrawn from each beaker separately. The decrease in solution volume was compensated with 3 mL fresh phosphate buffer solution to keep the volume constant at 20 mL. Samples were withdrawn with compensation for the loss at 0.25h, 0.5h, 1h, 2h, 3h, 4h, 6h, 12h and 24h. Absorbance was measured for the solutions that at 230 nm wavelength. The concentrations were calculated utilizing the calibration curve, followed by a series of calculations to obtain the release percentages as shown in section (2.7).

2.6 Characterization

2.6.1 Fourier Transform Infrared (FTIR) Analysis

Chemical structure determination and the investigation of the interactions between chitosan and with the other components in the membrane, was carried out using Mattson Satellite 5000 FTIR spectrophotometer. The samples were analyzed in the membrane form by placing the membrane as it is in FTIR instrument cell, while the pure substances (for comparison) are used as powder and mixed with potassium bromide powder.

2.6.2 Ultraviolet-Visible (uv-vis) Spectrometry

The model of uv-vis spectrometer T 80 + UV\VIS Spectrometer PG Instrument Ltd was used.

2.6.3 Scanning Electron Microscope (SEM) Analysis

The morphology of the surface for each membrane had been investigated by using SEM analysis. This analysis had been done at TUBITAK-MAM in Turkey.

2.7 Calculations

2.7.1 Swelling Percentage

The swelling percentage for each membrane was calculated accordingly:

$$\%swelling = \frac{w_2}{w_1}\ x\ 100 \quad (2.1)$$

Where:

w_1 = weight of the membrane before soaking in water

w_2 = weight gain of the membrane after soaking in water (for each given time interval).

2.7.2 Naproxen Loading Percentage and Loading Efficiency

As mentioned above, naproxen solution was prepared by dissolving naproxen 0.0156 g in 100 mL phosphate buffer solution (pH 7.4). Each membrane was soaked separately in 25 mL of this solution for 30 minutes; this experiment was repeated twice. The amount of naproxen in 25 mL solution was calculated as below:

$$\frac{25mL*0.0156g}{100} = 0.0039g$$

For the weight of naproxen which had been loaded in to each membrane:

$$W = W_1 - W_0 \quad (2.2)$$

Where:

W: Weight of loaded naproxen in to the membrane

W_0: Weight of the membrane before loading naproxen

W_1: Weight of the membrane after loading naproxen

Loading efficiency (% D) was calculated accordingly:

$$\%D = \frac{W}{0.0039} * 100 \quad (2.3)$$

The percentage of the naproxen loaded into each membrane (P) calculated by:

$$\%P = \left(\frac{W}{W_0}\right) * 100 \quad (2.4)$$

2.7.3 *In-vitro* Release Percentage

The drug release percentage for each membrane obtained at given time intervals. Each experiment denoted with a number *y*, where *y* = 1, 2, 3… etc. the number *y* = 1 refers to the number of experiment for getting release percentage at 0.25 hour, when *y* = 2 refers to the number of experiment for getting release percentage at 0.5 hour and when *y* = 3 refers to the number of experiment for getting release percentage at 1 hour, and so on.

The absorbance which was obtained by uv-visible analysis after release was applied on the calibration curve's equation. The Beer's-Lambert curve gave the unknown concentrations of naproxen which was released from the sample for each given time. The equation for the calibration curve had been got from Microsoft Excel program as:

$$A_y = 0.0976 * M_y + 0.0347 \quad (2.5)$$

Where:

A_y: Absorbance

M_y: Concentration of naproxen

$$\mathrm{M}_{\mathrm{released}} = M_y - \bar{M}_{y-1} \quad (2.6)$$

Where:

$\mathrm{M}_{\mathrm{released}}$: Molarity of the drug released in experiment *y*

$\bar{M}_{y-1}$: Molarity of the drug after drawing a given volume of sample and compensating with fresh solvent.

$$n_y = M_y * V_L \quad (2.7)$$

Where:

n_y: Number of moles of drug in solution in experiment y

V_L: Volume in liters of release medium and it is always constant ($20*10^{-3}$ liter)

$$n_{released} = M_{release} * V_L \quad (2.8)$$

Where:

$n_{released}$: Number of moles of drug released

$$\bar{n}_y = n_y - (M_y * v_L) \quad (2.9)$$

Where:

$\bar{n}_y$: Number of moles of the drug after pulling 3 mL for analysis

v_L: Volume in litter which pulled for analysis and it is always constant ($3*10^{-3}$ liter)

$$\bar{M}_y = \frac{\bar{n}_y}{V_y} \tag{2.10}$$

$$W_{released\ y} = n_{released} * molar\ mass\ of\ the\ drug\ (naproxen)$$

Where:

$W_{released_y}$: Weight of the drug released in experiment y

$molar\ mass\ of\ the\ drug\ (naproxen)$ = 230.26 g/mole

$$W_{Total} = W_{released\ y} + W_{released\ y-1} \tag{2.11}$$

$$\%\ release = \frac{W_{Total}}{W_L} * 100 \tag{2.12}$$

Where:

$\%\ release$: release percentage

W_L: Weight loaded into each membrane from equation (2.2).

Chapter 3

RESULTS AND DISSCUSION

3.1 Chitosan Citrate Membrane Preparation

In this thesis, all the membranes which were formed from the polymer chitosan had been crosslinked with citrate solution. The degree of crosslinking can affect the swelling and dissolution properties of the chitosan chains. This in turn, can affect the release profile of all of these membranes, and it can reinforce the mechanical properties as well.

Ten samples of chitosan citrate membranes were prepared. The method for crosslinking for all of them was the same by soaking these membranes in 2.5% trisodium citrate solution for 48 hours at 4°C, and drying them at room temperature as mentioned in the experimental chapter section (2.3). Crosslinking was the final stage of the preparation of all of these membranes. The concentration of chitosan in six samples (sample 1, 2, 3, 4, 5, and 6) was 0.5% w/v, while for the remaining four samples (7, 8, 9, and 10) this concentration was 1% w/v. In (Figure 5) optical pictures have been presented. It can be observed that the concentration of chitosan effects on the transparency of the membrane. Membranes cast from chitosan solution of lower concentration (0.5% w/v) are more transparent when compared to the others (1% w/v).

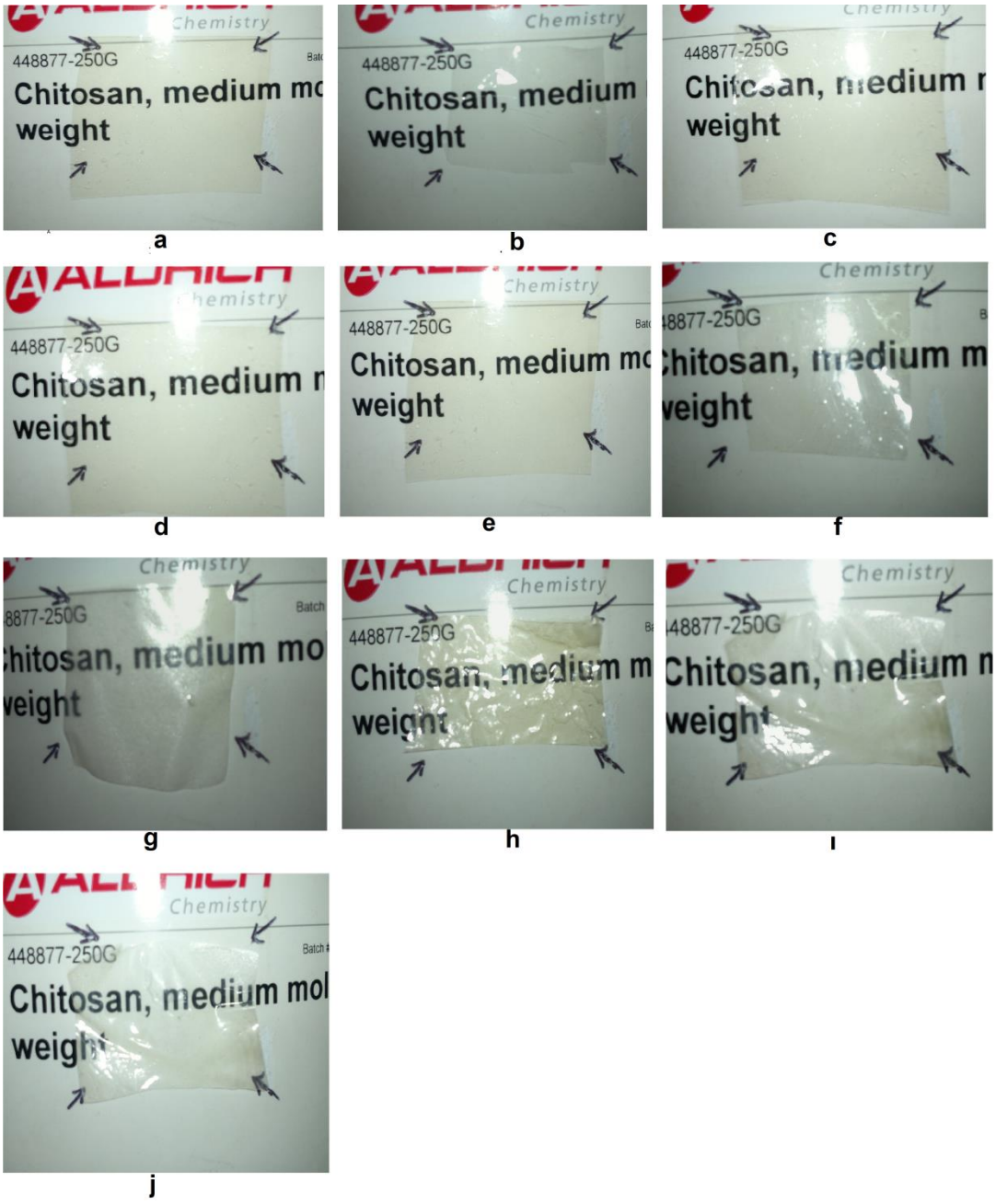


Figure 5: a, b, c, d, e, f, g, h, i and j are photographic pictures for samples 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 respectively

The average thickness of the films prepared from the more concentrated solution is higher than that of the ones obtained from lower concentration solution. Forthermore, soaking in NaCl causes a notable decrease in thickness. The average film thickness values are shown in (Table 2). The observations made on the films prepared and their optical pictures have been presented above. Further characterizations by SEM and FTIR analyses will be discussed in the following sections.

Table 2: The Average Thickness for the Ten Samples.

Sample	Average thickness/ mm
1	0.027
2	0.005
3	0.018
4	0.030
5	0.020
6	0.030
7	0.100
8	0.055
9	0.045
10	0.020

3.1.1 Membrane Modification by PEG

The addition of polyethylene glycol PEG, a low molecular weight non-toxic and biocompatible polymer to chitosan solution, as another polymer was conducted on the following samples 3, 4, and 6 in a ratio of 1/8 (w/w) of the weight of chitosan. Sample

5, on the other hand, contains PEG in the ratio 1/4 W/W with respect to the weight of chitosan. This polymer was added to improve the mechanical properties. PEG is known to bring in elasticity to polymer films [65]. It may also affect the surface morphology, swelling and release properties. This addition showed somewhat noticeable effects on transparency by make the membrane less transparent (Figure 5). Also, it showed effects on morphology of the surface of the film according to SEM analysis, in addition to the effects that had emerged on the swelling of the membranes or release state of naproxen. These effects will be addressed in the following sections.

3.1.2 Membranes Modification by Glycerol

Similar to PEG, glycerol had been used to improve membrane elasticity. Examples available in the literature show that including glycerol in the membrane composition improves the mechanical properties. Choice of glycerol was due to its safety, biocompatibility characteristics [66]. Ten grams of glycerol had been added to chitosan solution of sample 8; this addition gave this sample more flexibility. Sample 8 as shown in (Figure 5-h) got its yellowish colour due to the presence of glycerol.

3.1.3 Introducing Porosity

Two procedures had been applied for the purpose of obtaining the porous membranes both are based on using NaCl. The choice of this salt was due to its low cost, safety and biocompatible properties. The first procedure was by soaking the dry membrane in high salty water solution (35% w/v), and then carrying out the cross-linking process as explained in experimental chapter section (2.3). This procedure had been applied for sample 2, sample 4, sample 6 and sample 10.

The second method was by using NaCl as porogen agent by adding the fine salt crystals to chitosan solution with high stirring until homogeneity was attained. After casting and drying, the membrane was washed with plenty of double distilled water to remove salt crystals. The final stage as usual was the crosslinking process. This procedure had been applied on sample 7 and sample 8 shown in (Figure 6).

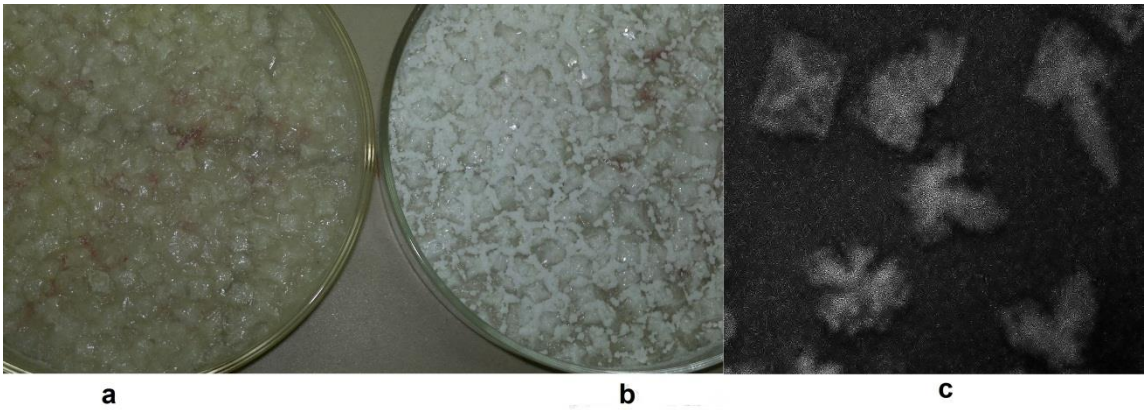


Figure 6: a-, b- shows conglomerates salt crystals on the surface of the membrane sample 8 and sample 7 respectively in one of the stages of the preparation of membrane before washing with double distilled and c- is zoom in for this crystallization.

3.2 Instrumental Analysis

3.2.1 FTIR Analysis

3.2.1.1 FTIR Analysis for Chitosan Powder

The FT-IR spectrum for pure chitosan powder showed the N-H bending peak at 1649 cm^{-1}.,while, the band for the stretching N-H overlapped with O-H stretching at 3449 $cm^{1.}$ The band of C-H stretching bands was located at 2923 cm^{-1}. On the other hand, the vibration of the C-O stretching in C6, which is considered as primary alcohol, appeared at 1080 cm^{-1}. Finally, the C-O-C stretching peak was located at 1377cm^{-1} (Figure 7).

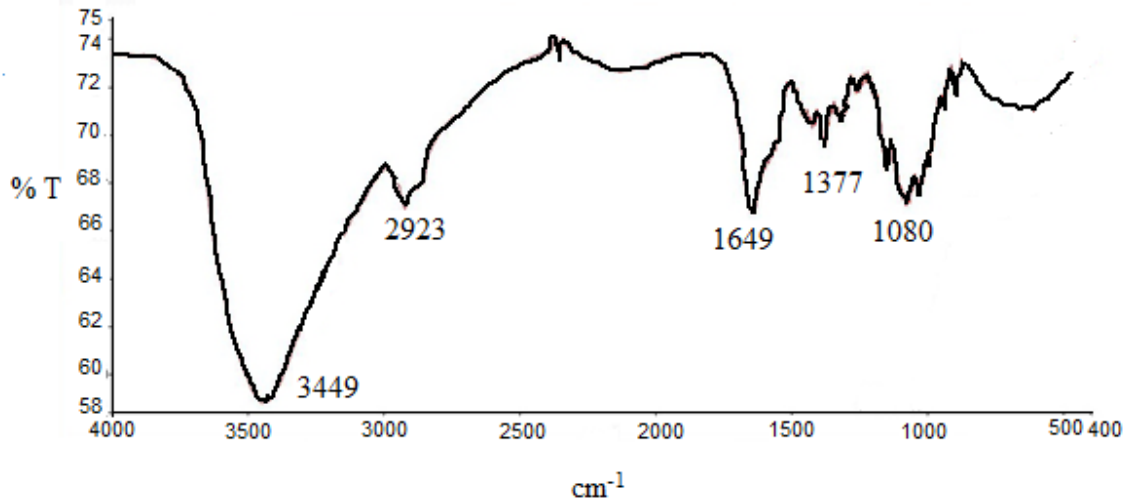


Figure 7: FTIR Spectra of chitosan pure powder

3.2.1.2 FTIR Analysis for Sodium Citrate Tribasic Dehydrate Powder

For this pure salt the FTIR analysis shows stretching for the three carboxylic OH a broad band at 3451 cm^{-1}. Another broad band appeared at 1591 cm^{-1} refer to the stretching of the carboxylic C=O, whereas the C-O stretching appeared at 1306 cm^{-1}. For the peak which located at 2924 cm^{-1} refers to the stretching of C-H bond (Figure 8).

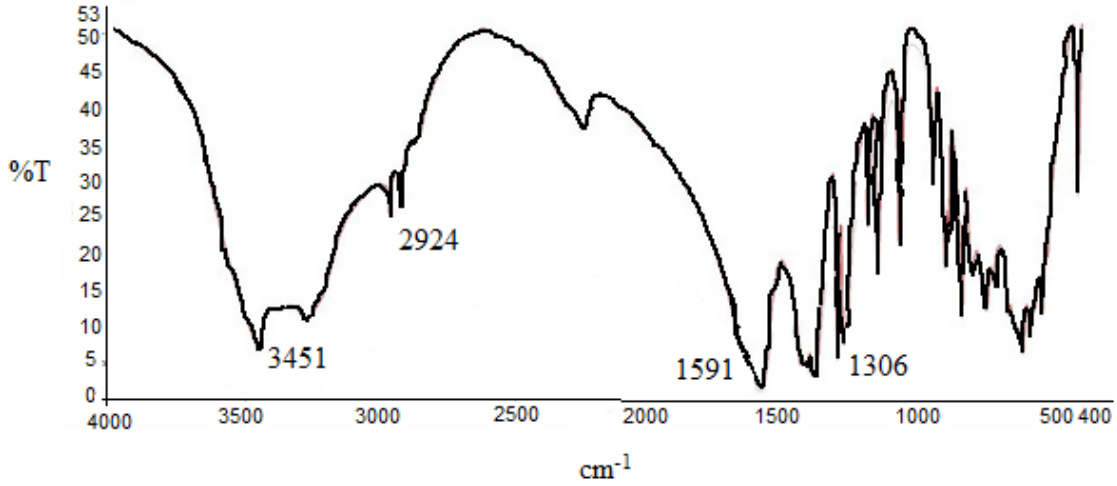


Figure 8: FTIR Spectra of Sodium Citrate Tribasic Dehydrate Pure Powder

3.2.1.3 FTIR Analysis for Chitosan Citrate membranes

All the membranes, which were prepared for this thesis work, had been treated with sodium citrate tribasic dehydrate (citrate solution) for crosslinking purposes. Sample 1 (Figure 9) and sample 9 (Figure 10) are chitosan citrate membranes which exhibit the interaction between chitosan and the citrate ion. A peak which appeared at 1654 cm^{-1} refers to the C=O of the salt which formed after the ionic interaction occurred between chitosan and citrate. Also peak appeared according to the symmetrical stretching of carboxyl group this band near 1400 cm^{-1}. Moreover, very broad band at 3383 cm^{-1} refers to the overlapping of OH group peak of citrate ion with the OH group peak of chitosan. This broad band didn't appear in the pure materials, neither chitosan nor citrate, as broad as it is in all samples of chitosan citrate membranes. The above spectroscopic peaks indicate the interaction between the protonated amine groups of chitosan (NH_3^+) with the carboxyl groups of the citrate (COO^-), and this crosslinking had been done for the ten samples. The other peaks which appear in the spectrum of these samples, existed almost same in the pure chitosan and citrate spectrum, and have already been interpreted in the above sections (Figure 9).

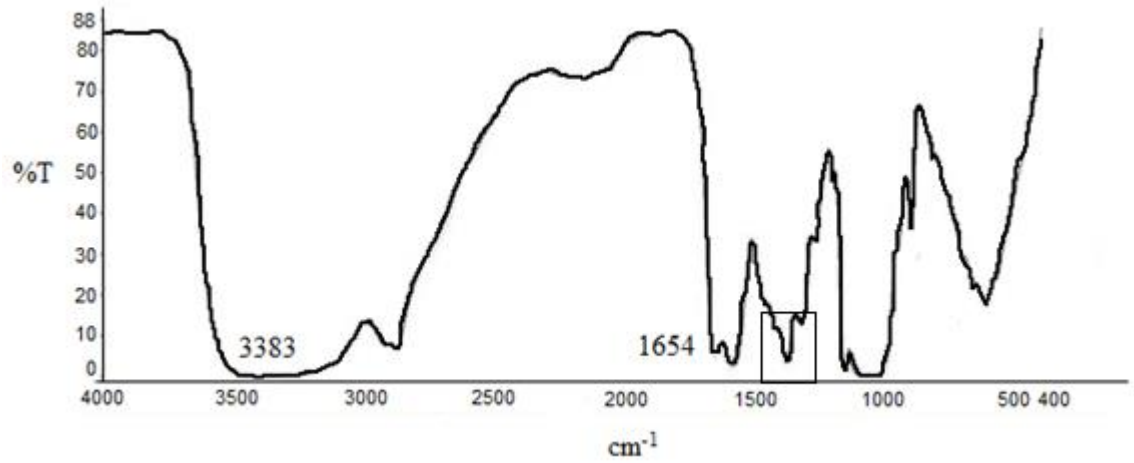


Figure 9: FTIR Spectra of Chitosan Citrate Membrane Sample 1

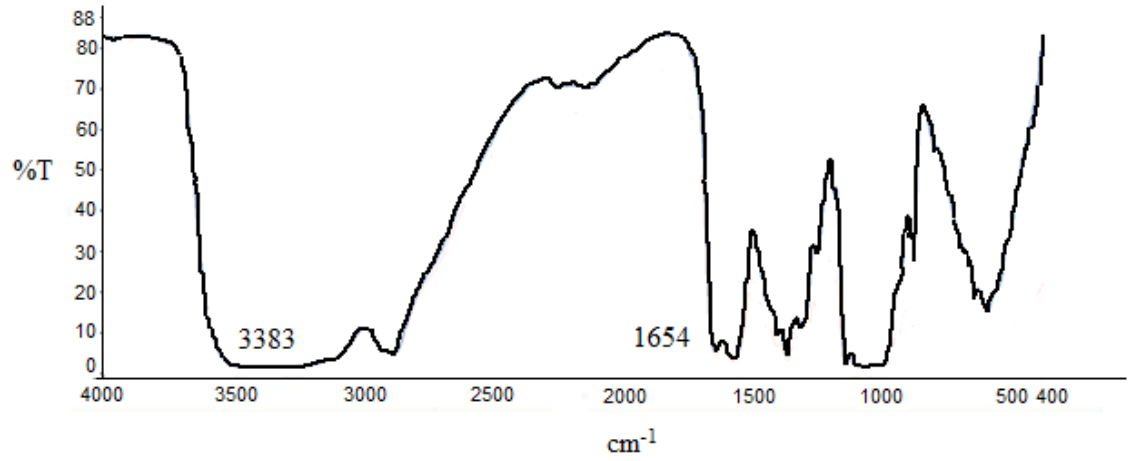


Figure 10: FTIR of Chitosan Citrate Membrane Sample 9

3.2.1.4 FTIR Analysis for PEG Powder

The FTIR for pure sample of PEG (Figure 11) shows OH stretching at 3427 cm^{-1}. Another peak appeared at 2884 cm^{-1} refers to stretching of C-H, while the scissoring and bending the same bond appeared at 1408 cm^{-1}. Finally, C-O peak and C-O-C vibration appeared at 1240 cm^{-1} and 1106 cm^{-1} respectively.

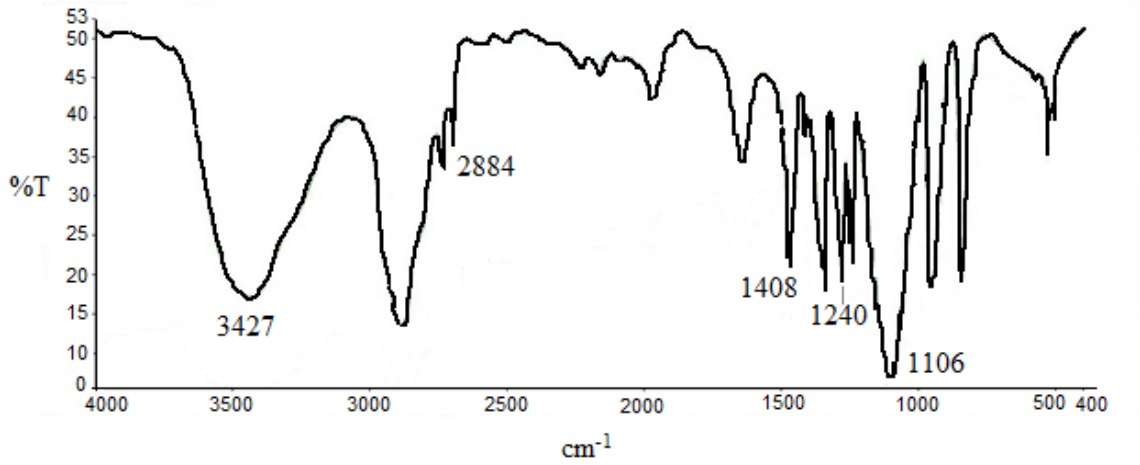


Figure 11: FTIR Spectra of PEG Pure Powder

3.2.1.5 FTIR Analysis for Chitosan Citrate Membranes Containing PEG

The samples, which have polyethylene glycol (PEG), were samples 3, 4, 5 and sample 6 for the FTIR analysis sample 3 and sample 4 were chosen due to their transparency appearance for more obvious peaks. Sample 3 will be taken as an example for comparing the samples with and without PEG (Figure 12). This sample has the same fraction of chitosan and all the conditions of preparation as sample 1, but the last one was without PEG. For comparing with sample 1 in (Figure 9) all the peaks appeared around same ranges, because there is no new functional group added with PEG addition, but the peaks shape in case of sample 3 is more sharper especially at the rang of the C-H bending and C-O, according to enhance the appearance of the same functional groups but more strong peak. This explanation reinforced by comparing the FTIR of these two samples with the FTIR of the pure PEG powder (Figure 11).

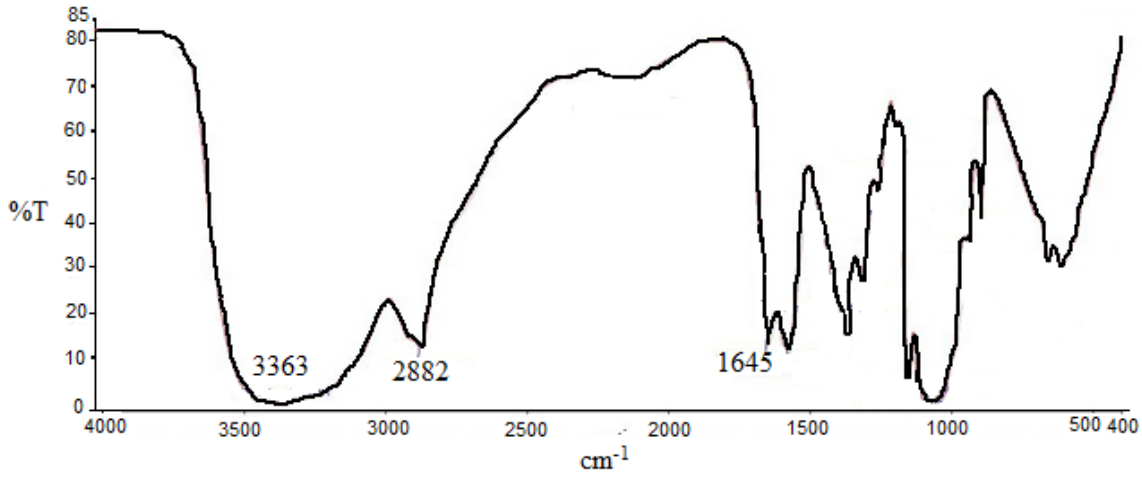


Figure 12: FTIR Spectra of Sample 3 which contain PEG

3.2.1.6 FTIR Analysis for Chitosan Citrate Membrane Contain Glycerol

The membrane which contains glycerol is sample 8. This sample has the same fraction of chitosan and all the conditions of preparation as sample 7, but the last one was without glycerol. In sample 8 a sharp peak appeared at 1379 cm^{-1} refers to the stretching of three C-O bonds in glycerol (Figure 13). The peak which appeared at 1418 cm^{-1} doesn't appear in sample 7 according to the literature it refers to the C-O-H bending of glycerol [64].

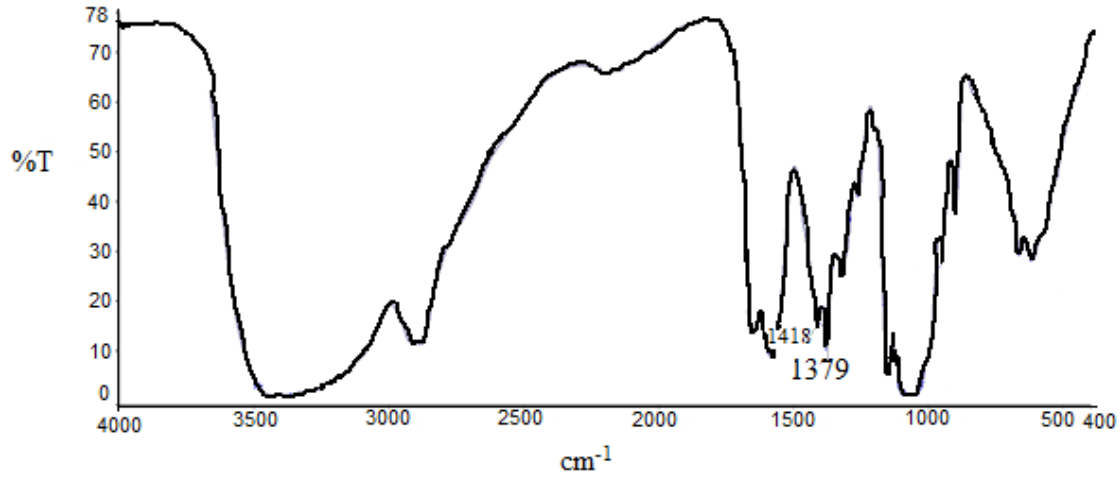


Figure 13: FTIR Spectra of Sample 8 which Containing Glycerol

3.2.1.7 FTIR Analysis for Naproxen Powder

Naproxen as pure sample shows FTIR peaks as, OH carboxylic at 3196 cm^{-1} and C-H aliphatic at 2975 cm^{-1}, while the aromatic C-H appeared at 3003 cm^{-1}. A sharp peak appeared at 1728 cm-1 it refers to C=O acidic, while the stretching of C-O appeared at 1264 cm^{-1}. The sharp peak which appeared at 1604 cm^{-1} refers to the presence of C=C alkene conjugated bond [67]. For benzene ring in the structure of naproxen, it shows a very sharp peak at 1604 cm^{-1} according to the C-C aromatic skeletal stretching. In addition to C=C aromatic bond which also appeared as a sharp peak at 1452 cm^{-1}.

Finally, the other sharp peak which appeared less than 1000 cm^{-1}, they are related to the aromatic ring bending (Figure 14).

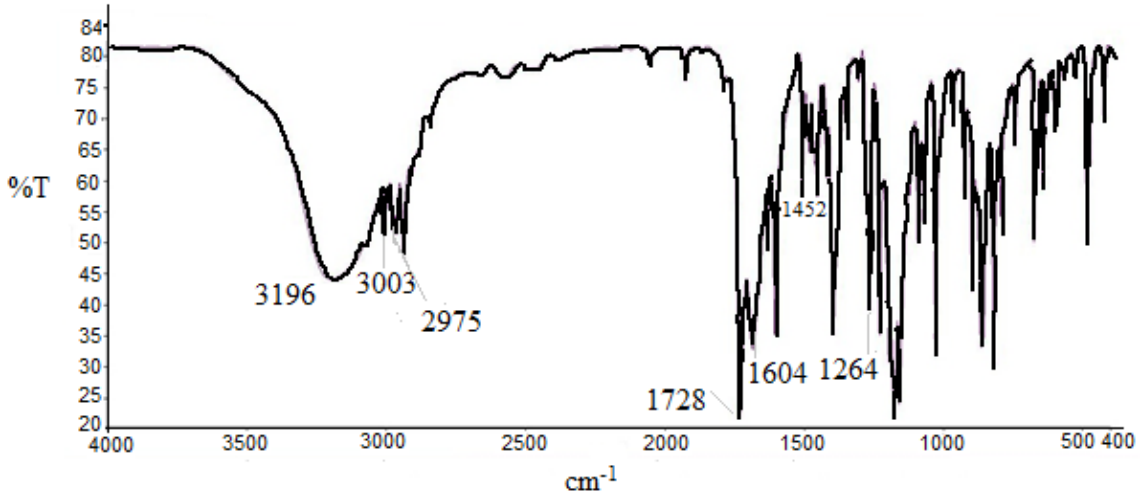


Figure 14: FTIR Spectra for Pure Naproxen Powder

3.2.1.8 Chitosan Citrate Membrane Loaded with Naproxen

The samples which had been loaded with naproxen as mentioned in experimental chapter entrapping naproxen section were samples 1, 3, 4, 7, 8 and 9. For showing the interaction between those membranes and naproxen, sample 1 had been chosen. Spectra of the sample 1 before and after loading with naproxen were compared to each other. A broad band appeared between 1411cm^{-1} till 1643 cm^{-1} in naproxen loaded membrane which refers to the stretching C=C conjugated bond which located in naproxen structure. The shift which happened at the OH region from 3383 cm^{-1} to 3418 cm^{-1} and it is broad appearance after loading naproxen. This broad peak refers to the overlapping of OH carboxylic which located in naproxen with the OH groups which already existing in chitosan citrate membranes alike. The peak which appeared at 792 cm^{-1} in naproxen loaded membrane is referring to the presence of C=C aromatic, as well as the other peaks which less than this range are related to the aromatic ring in naproxen structure.

This explanation may be tested by comparing the spectra of the two membranes (with and without naproxen) with the spectrum of pure naproxen powder (Figure 15)

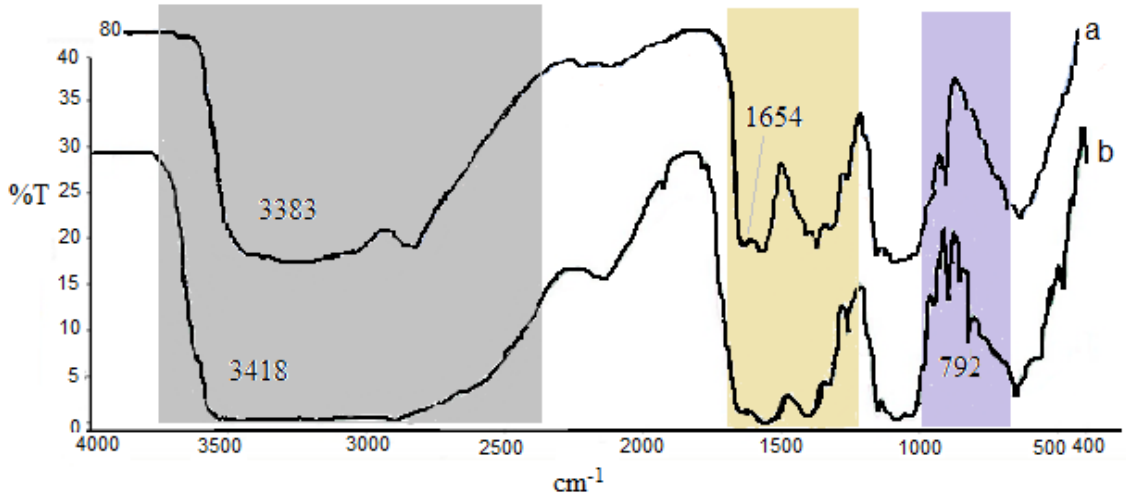


Figure 15: a- Spectra of Sample 1 Before Loading with Naproxen, b- Spectra of Sample 1 After Loading with Naproxen

3.2.2 SEM Analysis

Scanning electron microscopy analysis (SEM) had been applied on: sample 1, sample 2, sample 3, sample 4, sample 5, sample 6, sample 7, sample 8 and sample 9. The aim for using such analysis is to identify the morphology of surfaces of the membranes. Surface morphology is in terms of homogeneous and heterogeneous surfaces on one hand and in terms of porous and non-porous membranes on the other hand. These properties are strongly related to swelling of these membranes and release profile of naproxen from them.

3.2.2.1 SEM analysis for Non-Porous Membranes

Sample 1 and sample 9 which are considered as blank chitosan citrate samples look homogenous and smooth surface membranes, except the particles on the surface which

may refer to the presence of chitosan particles crystallizing on the surface of the membrane during casting. In addition to that, there are no pores that can be observed on this surface (Figure 16).

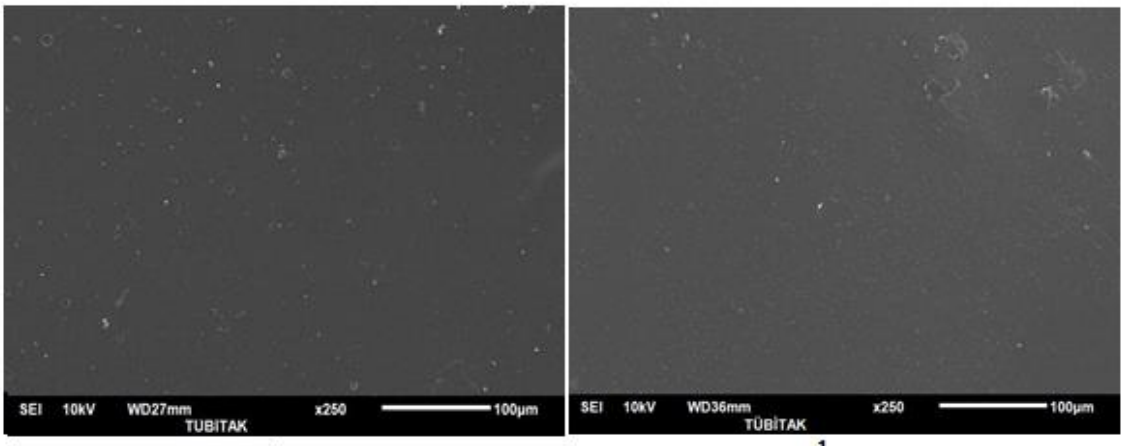


Figure 16: a- SEM Analysis for Sample 1 and b- SEM Analysis for Sample 9

In (Figure 17), sample 2 is the same as sample 1 in the method of preparation and materials ratio, but it is soaked in NaCl solution. There is no effect of soaking in NaCl on the morphology of surface (i.e.: sample 2 has the same homogeneity as sample 1), except that it may have induced crystallization of chitosan salt on the membrane surface.

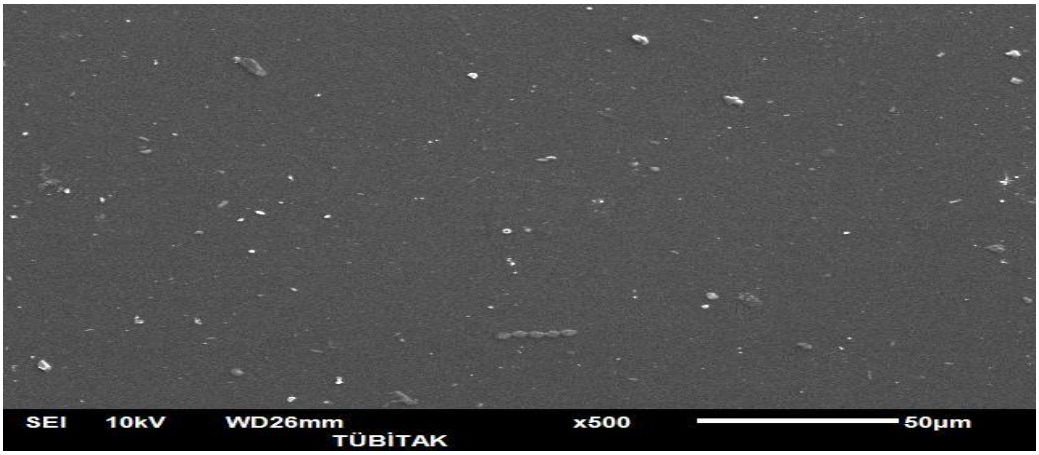


Figure 17: SEM Analysis for Sample 2

Sample 3 contains PEG in weight ratio 1/8 w/w of weight of chitosan. With this ratio there is an effect on the surface morphology it seems less smooth than the above samples as it seems in (Figure 18).

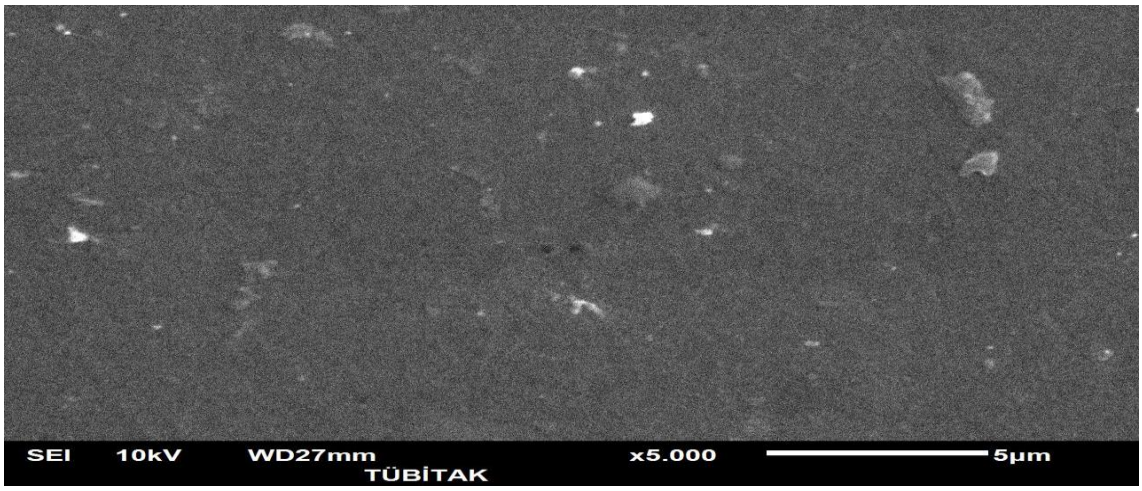
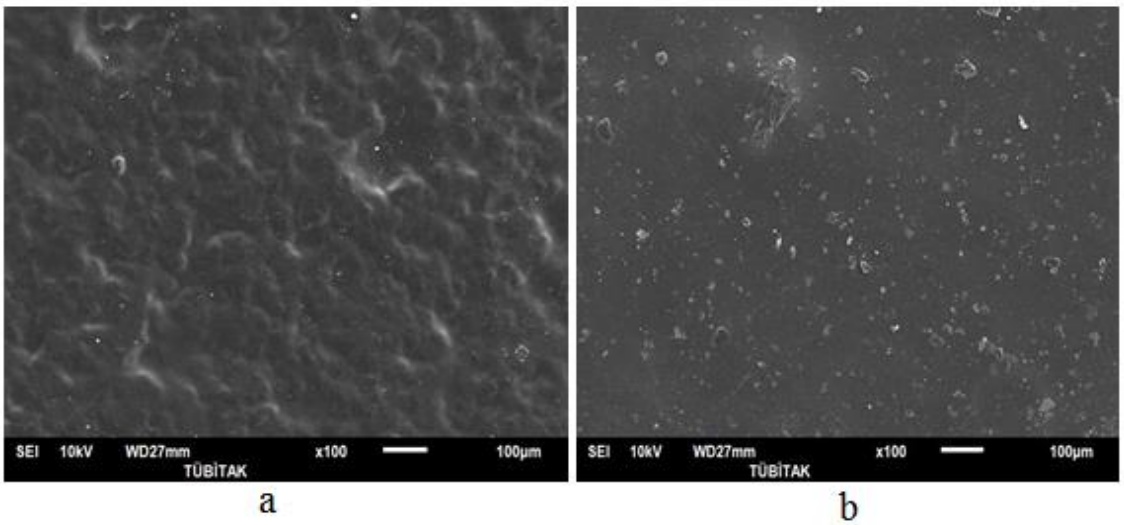


Figure 18: SEM Analysis for Sample 3

Sample 5 and sample 6 also contain PEG, but they have the weight ratio 1/4 w/w of weight of chitosan. This ratio can effect on the surface morphology and of the membrane as it seems in (Figure19). This differentiation is related to the high weight ratio of PEG if it is compared with sample 3 and sample 4 which has half of this ratio.



a b

Figure 19: a- SEM Analysis for Sample 5, b- SEM Analysis for sample 6

Some heterogeneous porosity can be observed in sample 4, which is same as sample 3 (contains PEG in weight ratio 1/8 W/W of weight of chitosan), but it is soaked in NaCl solution. This shows the effect of soaking in NaCl solution; it may give heterogeneous porous membranes in presence of PEG. Incompatibility and phase separation is observed at higher PEG ratio. Microdomains of PEG is dispersed in continuous chitosan phase (Figure 20).

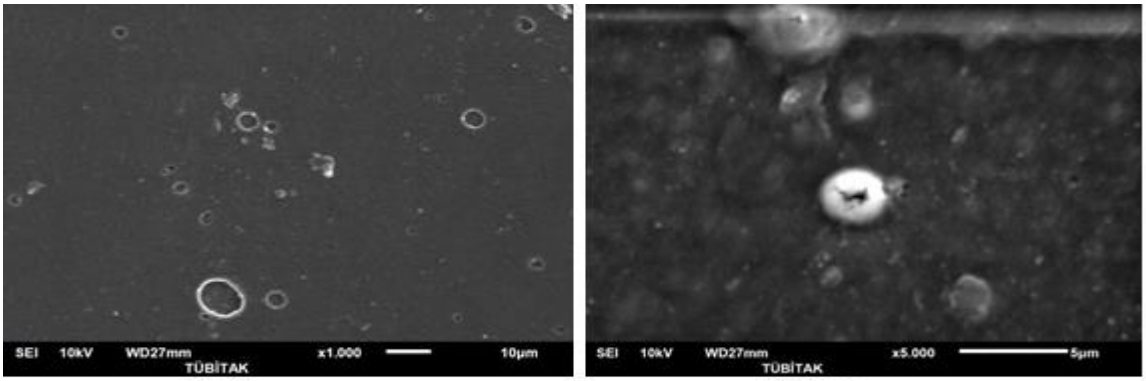


Figure 20: SEM Analysis for Sample 4 in two Different Views (a: *1000) and (b: *5000)

A higher degree of porosity can be observed in sample 7. The idea of using NaCl as porogen agent was more successful for forming porous membrane and it was better than soaking in NaCl solution for introducing porosity to the membrane (Figure 21).

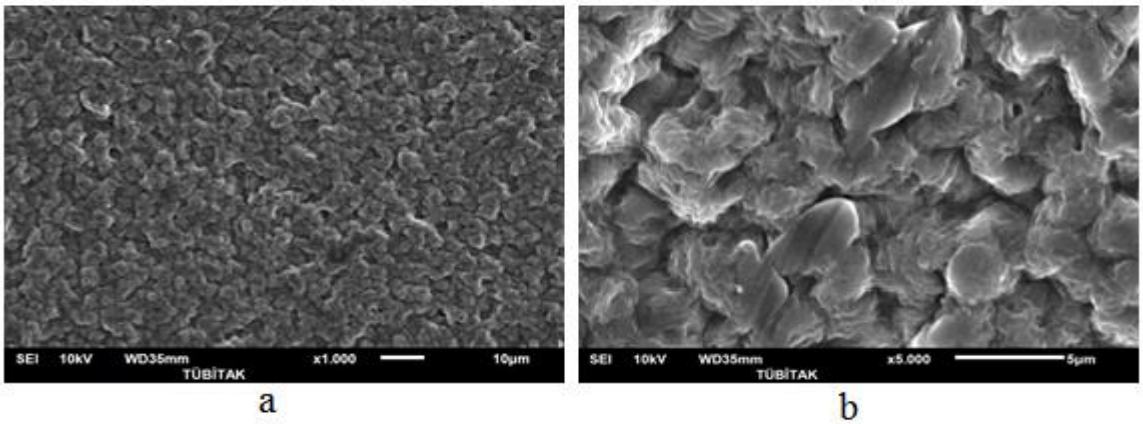


Figure 21: SEM Analysis for Sample7 in two Different Views (a: *1000) and (b: *5000)

In addition to sample 7, the same procedure was applied for sample 8 having glycerol. This sample also contained porosity, but not as homogenously distributed as in sample 7. The benefit of adding glycerol was that it gave better mechanical properties as can be tested by hand stretching. Sample 8 was more flexible than sample 7 and its flexibility can be observed from the bends of the chains as appeared in (Figure 22).

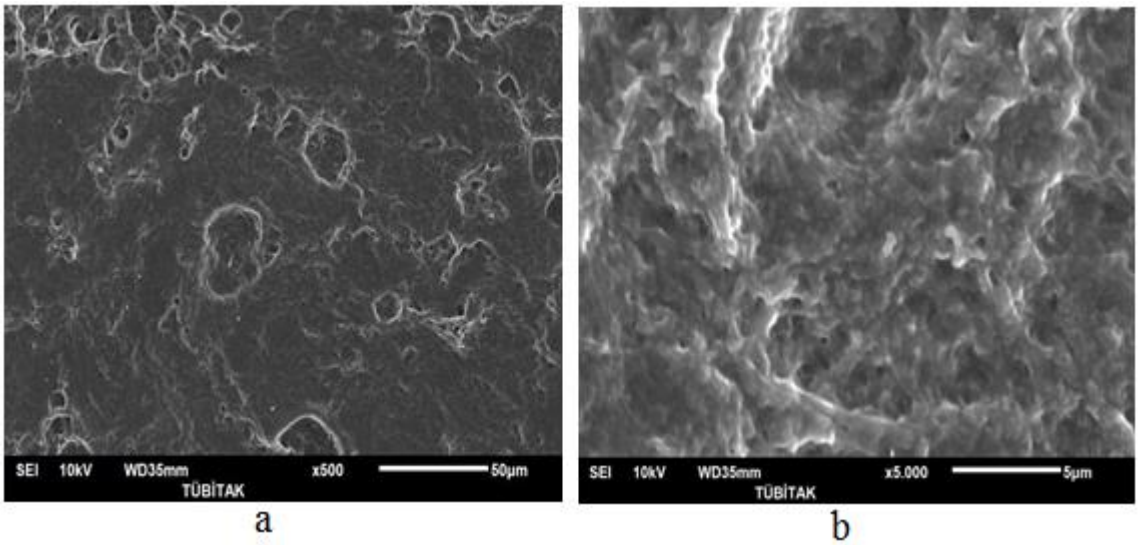


Figure 22: SEM Analysis for Sample 8 in two Different views (a: *500) and (b: *5000)

3.3 Swelling Study

In general, swelling behavior for the polymers is the first step of dissolving. For the polymer membranes, it can give information about the nature of this polymer, and release profile. The swelling study had been conducted for ten samples, and the behavior was different according to the chemical and physical properties of these membranes. The result of swelling percentage for this study for all of samples is shown in (Table 3).

Table 3: Swelling Percentages for Ten Membrane Samples

Time/ hours	Swelling Percentage %									
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10
0.25h	120	132	112	270	260	184	170	112	77	67
0.5h	112	140	285	172	265	198	220	155	98	85
1h	73	112	262	85	200	104	213	197	75	85
2h	42	172	255	180	221	173	193	202	102	123
3h	173	250	313	233	376	295	201	233	150	198
4h	180	222	352	223	322	342	248	218	150	196
5h	172	236	323	164	270	336	277	256	166	142
6h	169	243	290	210	244	267	276	302	140	148
18h	250	245	302	276	256	202	366	315	160	135
42h	215	235	316	243	255	217	370	333	143	136

The swelling percentages had been plotted and explained as given below. For sample 1 the swelling percentage showed increasing at first 15 minutes, and soon at 30 minutes started to decrease to reach to its lowest value 42% after 2 hours. After that the swelling percentage started to increase with fluctuating till reach to the peak after 18 hours which

reach to the highest value; 252%, and this percentage had not changed much after 42 hours (Figure 23-a). In sample 2 the meter wasn't too much different from sample 1, it showed same fluctuating in the first four hours, but the maximum swelling percentage was 245% after 18 hours then stabilized till 42 hours (Figure 23-b).

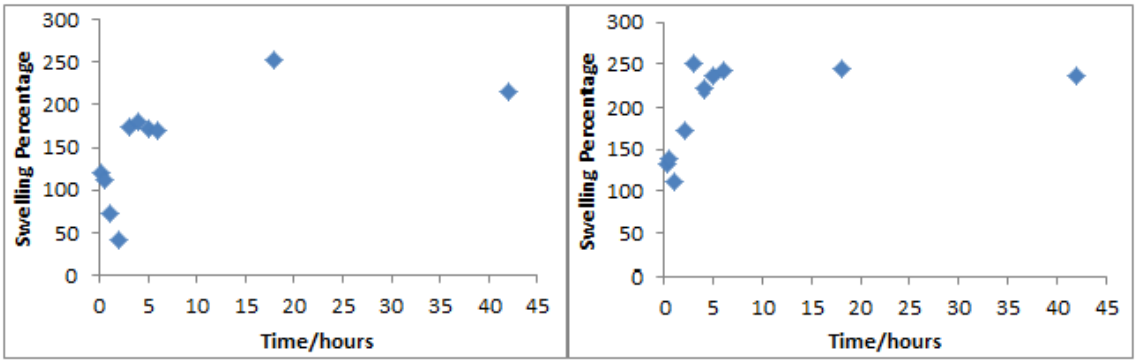


Figure 23: a- Swelling Behavior for Sample 1, b- Swelling Behavior for Sample 2

The swelling percentage for sample 3 rise after 30 minutes, while it decreased after one hour for the maximum value, it was 352% after 4 hours. Also after 6 hours the swelling percentage reached to constant value till 42 hours (Figure 24-a). For sample 4 showed a fluctuating at first two hours the release percentage reached to its maximum value 276% after 18 hours. And then swelling percentage had been stabilized and continued stable until 42 hours (Figure 24-b).

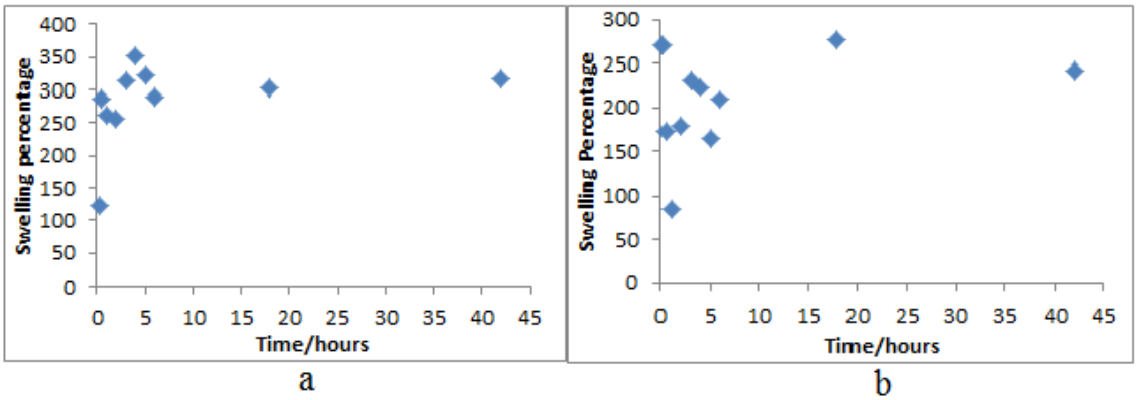


Figure 24: a- Swelling Behavior for Sample 3, b- Swelling Behavior for Sample 4

Sample 5 the same as other membranes had fluctuation till reach to the peak of swelling percentage 367% which is the highest swelling among all membranes after 3 hours. The stabilization of swelling percentage was after 5 hours and continued approximately same till 42 hours (Figure 25-a). Sample 6 has the maximum swelling percentage after 4 hours was 342%, and this percentage decreased to reach 217 after 42 hours (Figure 25-b).

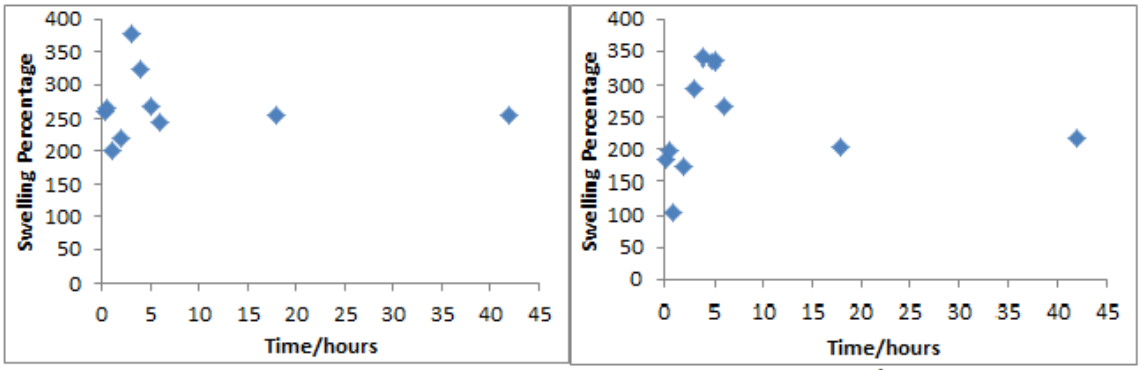


Figure 25: a- Swelling Behavior for Sample 5, b- Swelling Behavior for Sample 6

Sample 7 showed continuous rising with less fluctuation than the above samples membranes until 42 hours where was the peak of swelling percentage 370% (Figure 26-a). For sample 8 the behaviour was similar to sample 7 in terms of rise steadily. Also this sample showed a notable increasing in swelling percentage until reach to the peak 333% after 42 hours (Figure 26-b).

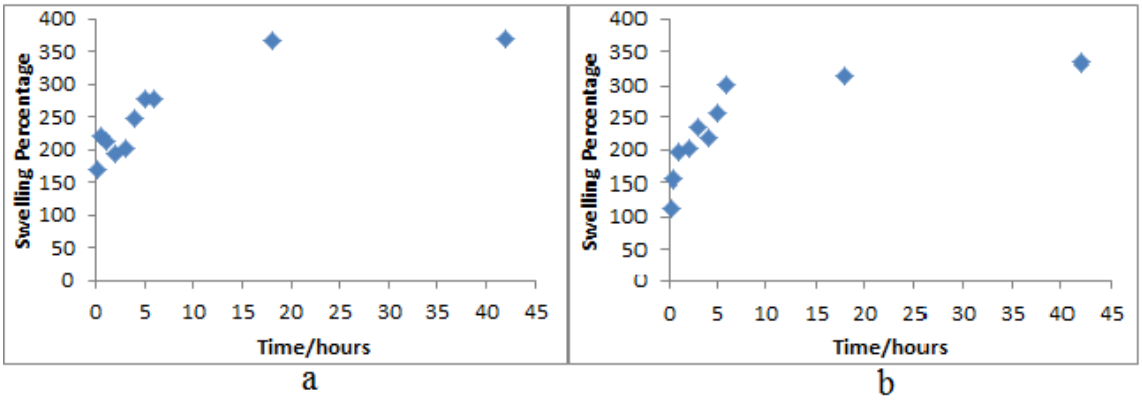


Figure 26: a- Swelling Behavior for Sample 7, b- Swelling Behavior for Sample 8

Sample 9 showed maximum swelling percentage 166% after 5 hours. Then the swelling percentage stabilized until 42 hours (Figure 27-a). Finally, sample 10 showed maximum swelling percentage 198% after 3 hours, and then decreased after 5 hours to settle down after that until 42 hours (Figure 27-b).

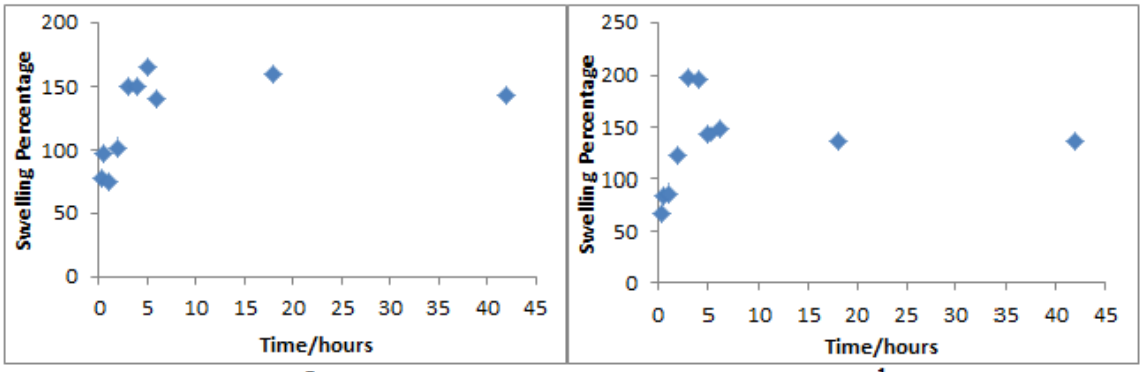


Figure 27: a- Swelling behavior for Sample 9, b- Swelling behavior for Sample 10

In general, the swelling behaviour for all of the membranes had been fluctuated this may be because of there is lose in the weight of the polymer due to dissolve and separate of low molecular weight pieces of the polymer. In addition, heterogeneities in physical crosslinking points affect osmotic pressure and this gave a wide range of changes in the swelling behaviour. The ratio of chitosan in the membrane also affected; if we compare sample 1 with sample 9, or sample 2 with sample 10 where the membranes that have 0.5% w/v of chitosan showed maximum swelling percentages higher than those that have 1% w/v of chitosan. It is worth mentioning that these pairs were only different in chitosan concentration. The reason behind this is due to the larger possibility of contacts of chitosan molecules due to less entanglement of polymer chains in case of 0.5% w/v of chitosan with water molecules. This higher contact with water is because of its less thickness and more homogenous (as showed in photographs above) than 1% w/v chitosan membranes and vice versa. When compared sample 1(which it is without PEG)

with sample 3 (which have PEG) it can be observed that the presence of PEG also gives the membranes higher swelling percentage than non-existence because the high ability of PEG to interact with water due to the hydrophilic nature of this polymer.

The membranes are classified above into five pairs and each pair contains same two membranes, but one of them with and the other without soaking in NaCl solution. Except the pair which had notable porosity (according to SEM analysis) which contains sample 7 and sample 8, the difference between them was the addition of glycerol to sample 8. The swelling percentage for most of these membranes shows a sharp decrease in swelling percentage a given time. As explained above this behaviour may relate to the dissolving of some parts of the polymer, and this effect on the weight measurement for that case.

Finally, sample 7 and sample 8 shows a steady rise in swelling percentage due to the presence of porosity on their surface according to SEM analysis.

3.4 Drug loading and drug release

3.4.1 Naproxen calibration curve

The absorbance of the concentrations which prepared (as mentioned in experiment chapter) had been measured by uv-visible spectrophotometer. The results are shown in (Table 4).

Table 4: Concentration versus Absorbance for Calibration Curve

Concentration in molar	Absorbance
$5 *10^{-5}$	0.552
$6 *10^{-5}$	0.665
$7.5 *10^{-5}$	0.785
$8 *10^{-5}$	0.824
$9 *10^{-5}$	0.875
$10 *10^{-5}$	0.984

The results applied in "Microsoft Excel" program by plotting concentrations versus absorptions the as shown in the plot below (Figure 28).

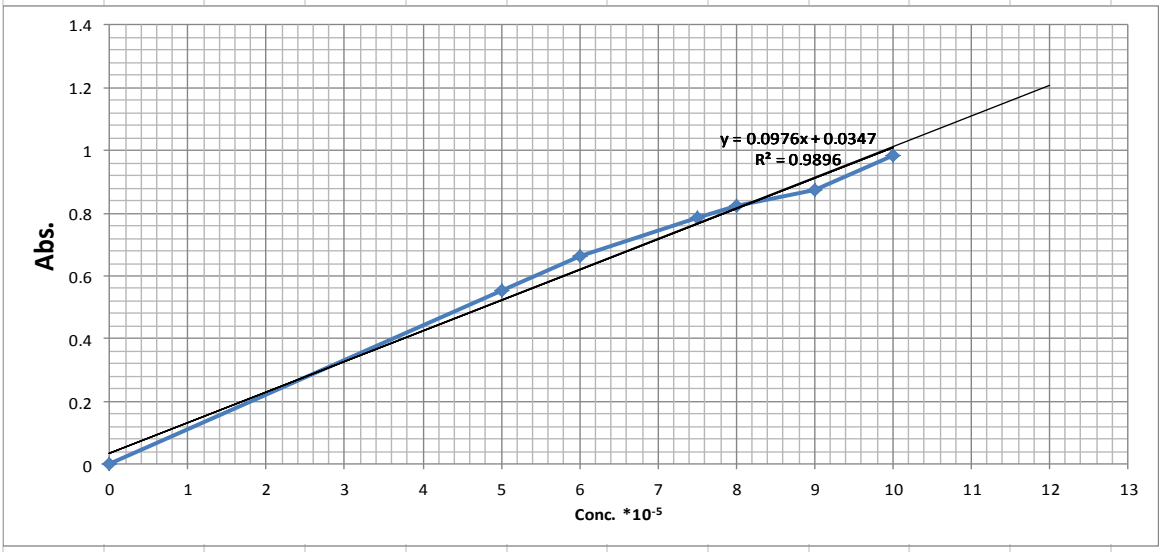


Figure 28: Naproxen Calibration Curve

3.4.2 Entrapping of naproxen (drug loading)

The samples which used for conducting drug loading-release study were sample 1, sample 3, sample 4, sample 7, sample 8, and sample 9. The results of Loading efficiency

loading and percentage as calculated in experimental chapter section (2.7) are shown in (Table 5).

Table 5: Loading Efficiency and Loading Percentage

Sample	Loading Efficiency (%D)	Loading Percentage (%P)
1	61.5	13.4
3	51.2	14.1
4	33.3	12.2
7	89.7	8.4
8	92.3	8.0
9	84.6	6.8

From the results of loading percentage, sample 8 was the membrane with the highest naproxen loading efficiency in which 92.3%/h drug has been loaded, followed by sample 7 which shows 89.7%/h. The reason behind these high loading percentages is the porosity that exists in these membranes according to SEM analysis. These pores gave a bigger surface area to the membrane, allowing the solution molecules that carry naproxen penetrating more inside the membrane, and thus trapping naproxen was more effective. It is worth noting that, the samples with 1% w/v chitosan ratio are more efficiency of loading than 0.5% w/v chitosan ratio. The reason may be due to the lack of swelling-shrinking fluctuation in case of 1% w/v chitosan as observed in swelling study.

Loading Efficiency shows different result from loading percentage where the 0.5% w/v chitosan ratio shows highest efficiency than those with 1% w/v chitosan ratio this may

related to the interaction with medium of the first one was more effective and vice versa. This can occur due to the highest chance of chitosan molecules in the membrane with ratio 0.5% w/v chitosan to interact with medium, because the presence of these molecules on the membrane surface was more than those with 1% w/v chitosan. This idea reinforced by the membrane thickness measurements of 0.5 % w/v chitosan in most cases was less than the thickness of the membranes with 1% chitosan ratio.

3.4.3 *In-vitro* release study (drug release)

The *in-vitro* release study had been done for the six samples as mentioned above. The medium of release study had been simulated conditions of the human body. This simulation performed by using phosphate buffer solution pH 7.4 (the human blood pH). Steady the temperature of the medium at 37 °C (human body temperature). This medium had been stirred by magnetic stirrer at 100 rpm for the duration of the experiment. It is worth mentioning that, phosphate buffer solution had been used in the literature for such pharmaceutical or biological studies. A series of calculations had been applied started from the absorbance which got for a given release time until getting the release percentage at that time, and this applied for six samples alike. The formulas were shown in experimental chapter section (7.2). The results which obtained for each membrane were shown in the below (Tables 6, 7, 8, 9, 10, 11) for sample 1, 3, 4, 7, 8, 9 respectively.

Table 6: Release Results for Sample 1 (W_L=24*10^{-4} g)

y	T/h	A_y	M_y *10^{-5}	$M_{released}$ *10^{-5}	n_y *10^{-5}	$n_{released}$ *10^{-5}	$\bar{n}_y$ *10^{-5}	$\bar{M}_y$ *10^{-5}	$W_{rele.y}$ *10^{-5}	W_{Total} *10^{-5}	% $release$
1	0.25	0.693	6.745	6.745	13.490	13.490	0.023	5.733	31.06	31.06	13.0
2	0.5	0.707	6.888	1.155	13.790	0.0231	0.117	5.862	5.32	36.36	15.20
3	1	0.670	6.509	0.647	0.1302	0.0129	0.111	5.534	2.98	39.33	16.45
4	2	0.641	6.212	0.678	0.1242	0.0135	0.105	5.275	3.12	42.45	17.68
5	3	0.602	5.812	0.537	0.1162	0.0107	0.098	4.940	2.47	44.92	18.71
6	4	0.566	5.443	0.818	0.1088	0.0163	0.092	4.625	3.78	48.70	20.29
7	6	0.467	4.429	0.649	0.0885	0.0129	0.075	3.760	2.99	51.69	21.53
8	12	0.301	2.728	0	0.0545	0	0.046	2.319	0	51.69	21.53
9	24	0.149	1.171	0	0.0234	0	0.019	0.995	0	51.69	21.53

Table 7: Release Results for Sample 3 (W_L=20*10^{-4})

y	T/h	A_y	M_y *10^{-5}	M_{releas} *10^{-5}	n_y *10^{-5}	$n_{released}$ *10^{-5}	$\bar{n}_y$ *10^{-5}	$\bar{M}_y$ *10^{-5}	$W_{rele.y}$ *10^{-5}	W_{Total} *10^{-5}	% $release$
1	0.25	0.616	5.955	5.955	11.910	0.119	0.101	5.061	27.42	27.42	13.71
2	0.5	0.598	5.679	0.615	0.113	0.012	0.096	4.825	2.83	30.25	15.12
3	1	0.676	6.570	0.986	0.131	0.019	0.111	5.584	4.54	34.79	17.39
4	2	0.521	4.982	0	0.099	0	0.084	4.235	0	34.79	17.39
5	3	0.498	4,746	0.511	0.094	0.010	0.080	4.034	2.35	37.14	18.57
6	4	0.462	4.378	0.344	0.087	0.006	0.074	3.721	1.57	38.70	19.35
7	6	0.303	2.748	0	0.054	0	0.076	2.335	0	38.70	19.35
8	12	0.274	2.451	0.116	0.049	0.002	0.041	2.083	0.53	39.23	19.61
9	24	0.281	2.523	0.440	0.050	0.008	0.042	2.147	2.02	41.25	20.62

Table 8 : Release Results for Sample 4 (W_L=13*10^{-4})

y	T/h	A_y	M_y *10^{-5}	M_{releas} *10^{-5}	n_y *10^{-5}	$n_{released}$ *10^{-5}	$\bar{n}_y$ *10^{-5}	$\bar{M}_y$ *10^{-5}	$W_{rele.y}$ *10^{-5}	W_{Total} *10^{-5}	% $release$
1	0.25	0.450	4.255	4.255	8.510	8.5100	0.072	3.616	19.59	19.59	15.6
2	0.5	0.468	4.439	0.823	0.088	0.0164	0.075	3.773	3.790	23.38	17.99
3	1	0.428	4.029	0.256	0.080	0.0051	0.068	3.425	1.181	24.56	18.89
4	2	0.416	3.906	0.481	0.078	0.0096	0.066	3.319	2.215	26.77	20.59
5	3	0.378	3.517	0.198	0.070	0.0003	0.059	2.989	0.091	26.86	20.66
6	4	0.367	3.404	0.415	0.068	0.0083	0.057	2.893	1.911	28.77	22.13
7	6	0.358	3.312	0.419	0.066	0.0083	0.056	2.815	1.929	30.69	23.61
8	12	0.346	3.189	0.374	0.063	0.0074	0.054	2.710	1.722	32.41	24.93
9	24	0.340	3.128	0.468	0.062	0.0009	0.053	2.660	0.215	32.62	25.09

Table9 ： Release results for sample 7 (W_L=35*10^{-4})

y	T/h	A_y	M_y *10^{-5}	M_{releas} *10^{-5}	n_y *10^{-5}	$n_{released}$ *10^{-5}	$\bar{n}_y$ *10^{-5}	$\bar{M}_y$ *10^{-5}	$W_{rele.y}$ *10^{-5}	W_{Total} *10^{-5}	% $release$
1	0.25	0.974	9.623	9.623	19.24	19.24	0.1635	8.175	44.31	44.31	12.66
2	0.5	0.874	8.599	0.424	0.171	0.0084	0.1461	7.305	1.95	46.26	13.21
3	1	0.798	7.820	0.515	0.156	0.0103	0.1329	6.647	2.37	48.63	13.89
4	2	0.793	7.769	1.122	0.155	0.0224	0.1321	6.604	5.17	53.79	15.37
5	3	0.706	6.878	0.274	0.137	0.0045	0.1169	5.840	1.26	55.05	15.72
6	4	0.668	6.488	0.648	0.129	0.1296	0.1103	5.515	2.98	58.03	16.58
7	6	0.631	6.109	0.594	0.122	0.0119	0.1039	5.193	2.76	60.76	17.36
8	12	0.702	6.837	1.644	0.136	0.0329	0.1162	5.811	7.57	68.33	19.52
9	24	0.704	6.857	1.046	0.137	0.0209	0.1165	5.825	4.81	73.14	20.89

Table 10: Release Results for Sample 8 (W_L=36*10^{-4})

y	T/h	A_y	M_y *10^{-5}	$M_{release}$ *10^{-5}	n_y *10^{-5}	$n_{released}$ *10^{-5}	$\bar{n}_y$ *10^{-5}	$\bar{M}_y$ *10^{-5}	$W_{rele.y}$ *10^{-5}	W_{Total} *10^{-5}	% $release$
1	0.25	0.608*	11.74	11.74	23.49	23.49	0.1996	9.984	54.09	54.09	15.02
2	0.5	0.560*	10.76	0.780	0.215	0.0156	0.1829	9.149	3.59	57.68	16.02
3	1	0.952	9.39	0.249	0.187	0.0049	0.1597	7.988	1.146	58.82	16.34
4	2	0.927	9.14	0.154	0.182	0.0230	0.1554	7.770	5.314	64.13	17.81
5	3	0.849	8.43	0.573	0.166	0.0114	0.1418	7.091	2.639	66.76	18.54
6	4	0.814	7.98	0.893	0.159	0.0179	0.1357	6.785	4.122	70.88	19.68
7	6	0.830	8.14	1.364	0.162	0.0272	0.1375	6.877	6.281	77.16	21.43
8	12	0.850	8.35	1.476	0.167	0.0295	0.1420	7.100	6.797	83.96	23.32
9	24	0.853	8.38	1.284	0.168	0.0257	0.1425	7.126	5.913	89.87	24.96

Table 11: Release Results for Sample 9 (W_L=33*10^{-4})

Y	T/h	A_y	M_y *10^{-5}	$M_{release}$ *10^{-5}	n_y *10^{-5}	$n_{released}$ *10^{-5}	$\bar{n}_y$ *10^{-5}	$\bar{M}_y$ *10^{-5}	$W_{rele.y}$ *10^{-5}	W_{Total} *10^{-5}	% $release$
1	0.25	0.723*	14.10	14.10	28.20	28.20	0.2397	11.98	64.95	64.95	19.68
2	0.5	0.665*	12.91	0.935	25.83	0.0187	0.2195	10.97	4.31	69.25	20.98
3	1	0.578*	11.13	0.163	0.222	0.0326	0.1892	9.46	7.51	76.75	23.25
4	2	0.572*	11.01	1.550	0.220	0.0310	0.1871	9.35	7.14	83.88	25.42
5	3	0.989	9.77	0.427	0.195	0.0985	0.1656	8.28	1.97	85.84	26.01
6	4	0.899	8.85	0.575	0.177	0.0115	0.1505	7.52	2.65	88.48	26.81
7	6	0.895	8.81	1.295	0.176	0.0259	0.1498	7.49	5.96	94.44	28.61
8	12	0.812	7.96	0.474	0.159	0.0948	0.1353	6.77	2.18	96.62	29.27
9	24	0.798	7.82	1.17	0.156	0.0234	0.1329	6.65	5.39	102.0	30.91

* : The absorbance got after dilution; this mean the solution diluted by take 2 mL from the solution and diluted by 2 mL from fresh phosphate buffer solution. This process applied to follow Beer–Lambert law: $A=\mathcal{E}bc$; (the absorbance in uv-vis should be less than 1). After that the concentrations of these absorbents have been multiplied by 2 for correction.

3.4.3.1 Naproxen Release Profile of each Membrane

According to the above section, after getting the release percentages, release profiles had been shown in plots given below. All membranes show a burst effect within the first hour except sample 7 which showed the burst effect during the first two hours. The release behavior of these membranes is a complex system in which both swelling of the polymer and dissolution and diffusion of the drug are factors which have to be considered. It was tested whether the samples obeyed zero order release kinetics according to:

$$W_t / W_0 = k\, t^n \qquad (3.13)$$

Where n=1

The release profile for sample 1 and sample 3 did not show a big difference similar to swelling behavior. Sample 1 and sample 2 released 21.5% and 19.3% of naproxen respectively after 6 hours and until 24 hours sample 1 and sample 3, they released 21.5% and 20.6% respectively. (Figure 29-a), (Figure 30-a) shows the release percentage for sample 1 and sample 3 respectively while (Figure 29-b), (Figure 30-b) shows rate of release (Linear rise up progress) for sample 1 and sample 3 respectively. The release rate was until 5 hours for sample 1 was 1.258%/h, while for sample 3 was 0.98%/h.

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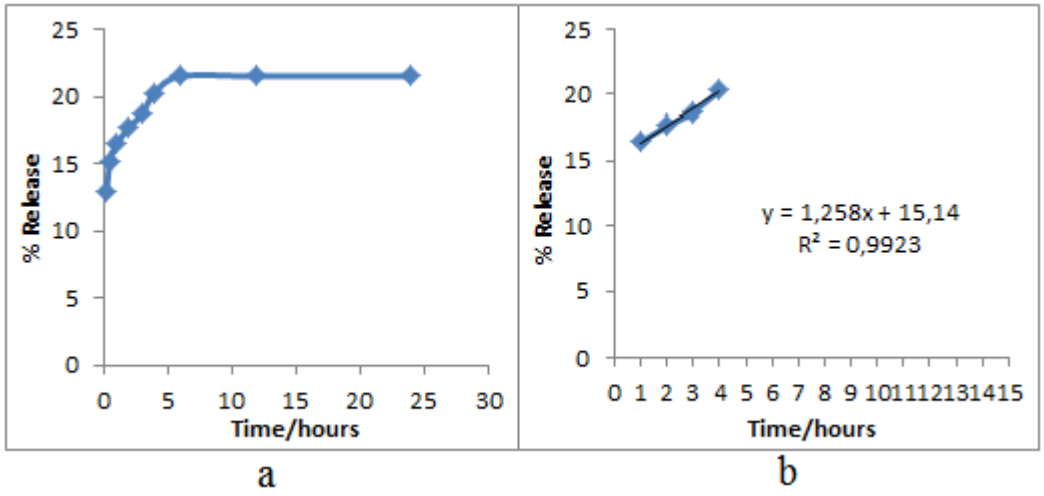

Figure 29: a- Release Percentage, b- Release Rate (linear rise up progress) for Sample 1

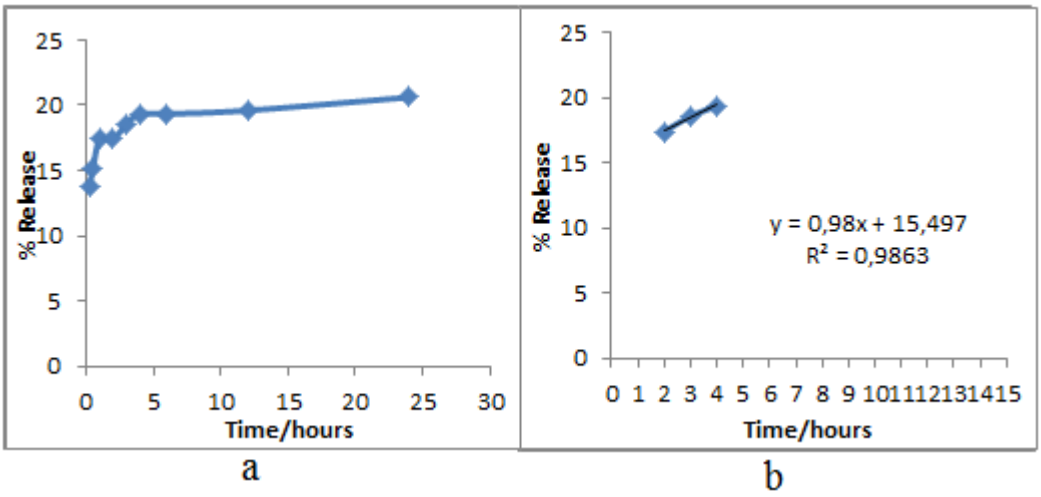

Figure 29 : a- Release Percentage, b- Release Rate (linear rise up progress) for Sample 3

For sample 4 it can observe that 23.6 % from naproxen had been released after 6 hours and 25% until 24 hours (Figure 31-a). For the release rate it was 0.9064%/h and there was a linear rise up progress until 6 hours (Figure 31-b).

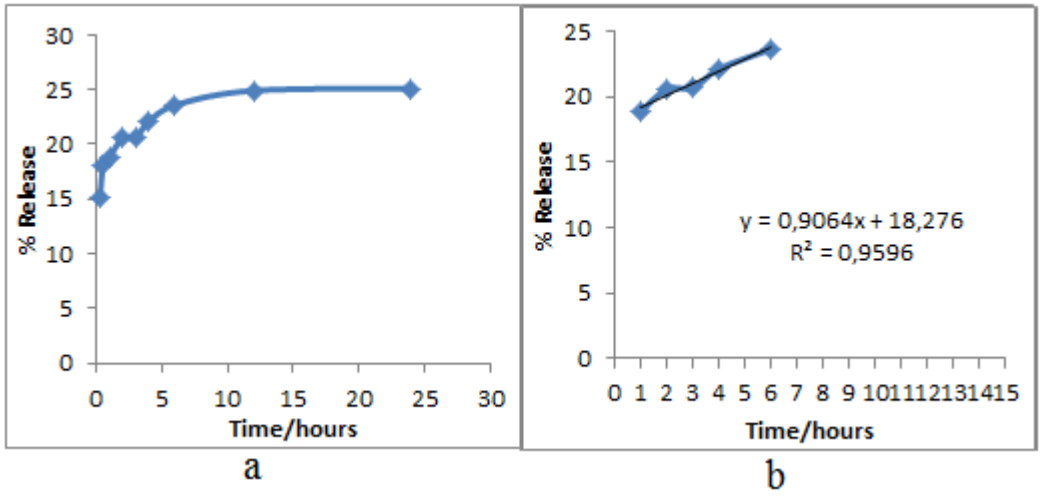

Figure 31: a- Release Percentage, b- Release Rate (linear rise up progress) for Sample 4

For sample 7 the release of naproxen was less than the other membranes, where just 17.3 % of naproxen had been release until 6 hours. In addition, this percentage did not increase significantly even after 24 hours, reaching to 20.8%. Sample 7 was considered (according to SEM analysis) as porous membrane; and the porosity might prolong the release of naproxen by obstructing motion of molecules out of this membrane (Figure 32-a). For the release rate of this membrane shows a linear rise up progress until 12 hours with release rate 0.4122%/h (Figure 32-b). It is worth mentioning this linear rise up progress can be observed in the swelling behavior for this membrane as well.

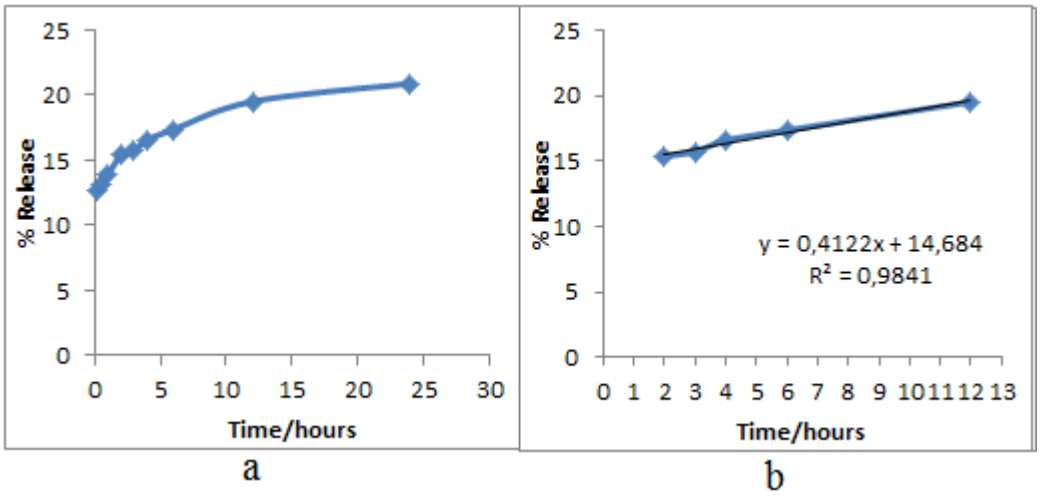

Figure 30: a- Release Percentage, b- Release Rate (linear rise up progress) for Sample 7

It seems to be different for the sample 8, which is also characterized by porosity (according to SEM analysis). This membrane shows 21.4% release of naproxen at the first 6 hours, while it reaches to more than 24.9% after 24 hours (Figure 33-a) . This might be related to the addition of glycerol which gave this membrane more flexibility than sample 7, if swelling behavior for this sample is taken into consideration. For the release rate it was 0.9946% and the linear rise up progress was until 6 hours (Figure 33-b).

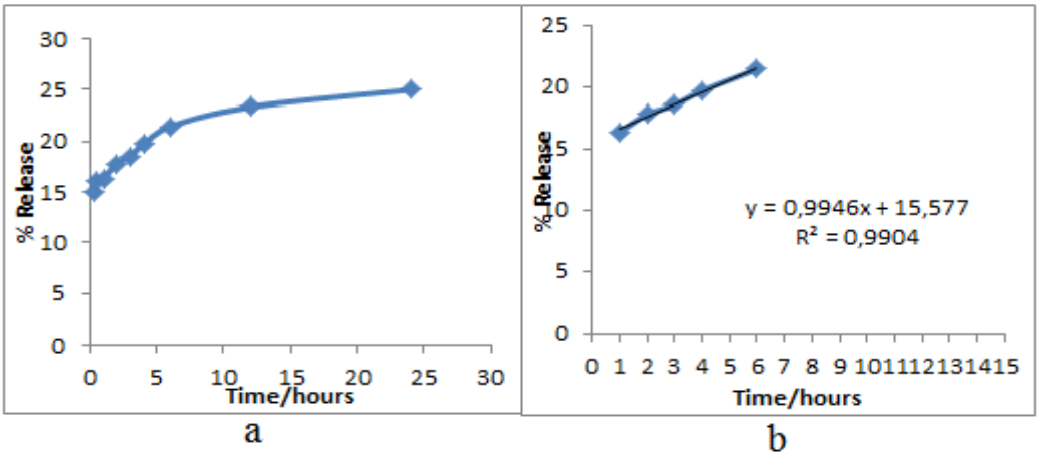

Figure 33: a- Release Percentage, b- Release Rate (linear progress) for Sample 8

Finally, Sample 9 according to SEM analysis was non-porous membrane this membrane released 28% of naproxen after 6 hours and this ratio had been increased until 24 hours to reach to 30.9% (Figure 34-a). For the release rate it was 0.9932% and the linear rise up progress was until 6 hours (Figure 34-b).

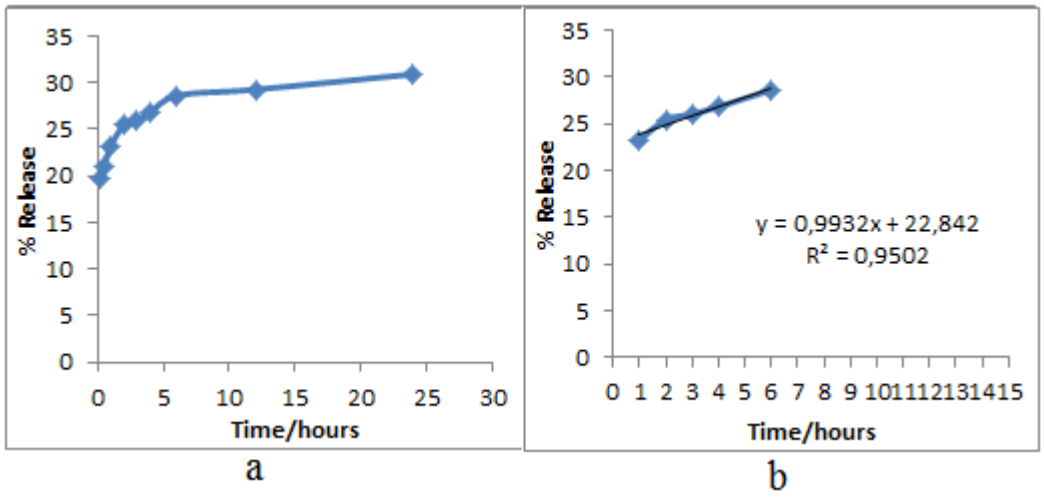

Figure 34: a- Release Percentage, b- Release Rate (linear rise up progress) for Sample 9

The release for all of the membranes showed a burst effect within 1 hour. Later they showed constant release within 1-6 hours (linear progress), except for the porous membrane sample 7 it was constant release from 2-12 h. For getting release rate the first data which are due to burst effect (sudden initial release) had been removed and the data at which they reach max release as well. The data in between should be linear to show rate of release which represents the slope of the line.

Chapter 4

CONCLUSIONS

1) Ten chitosan membranes have been prepared by solvent casting method for the purpose of finding a suitable drug delivery system for naproxen. The ratio of chitosan was 0.5% w/v for the six samples from 1 to 6, while the other four samples this ratio was 1% w/v. All of these membranes were crosslinked with citrate ion by the soaking method. The crosslinking was successfully achieved according to FTIR spectroscopy.
2) Four of these samples were blended with PEG for modification purposes; they were sample 1 and 4 with ratio 1/8 w/w of the weight chitosan. Sample 5 and 6 were modified with the ratio ¼ w/w of the weight of chitosan. These samples were studied for swelling and release behavior and were investigated by FTIR spectroscopy.
3) Sample 8 was modified by using glycerol as plasticizer. The effect of adding such plasticizer was studied by swelling and release behavior.
4) Introducing porosity to the membranes was carried out by two methods. The first one was soaking in NaCl solution which was not successful. The second method was by using NaCl as porogen which gave successful results when applied to sample 7 and 8. The porosity had been investigated by SEM analysis.

5) Swelling kinetics was followed for 42 hours in double distilled water at room temperature for ten samples. The membranes swelled to different degrees due to the effects of porosity and/or chemical content.
6) The maximum loading efficiency with naproxen was 92.3% for sample 8, while the maximum loading percentage was 14.1 for sample 3.
7) *In-vitro* release study was carried out for 24 in pH 7.4 phosphate buffer solution at 37°C. For samples 1, 3, 4, 8, 9, release rate was calculated at 1-6 hours, where there was a linear dependency of % release on time. After 6 hours, maximum % release was obtained which did not change until 24 hours. Sample 9 shows the highest release percentage with 31%. Sample 7 which contains porosity shows the longest linear release dependency on time from 2-12 hours reaching the maximum (21%) at 12 hours and remaining the same until 24 hours.

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